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# The BOTANICAL GAZETTE

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APRIL 1927

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Volume LXXXIII Number 2

# THE BOTANICAL GAZETTE

A JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

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*Issued April 21, 1927*

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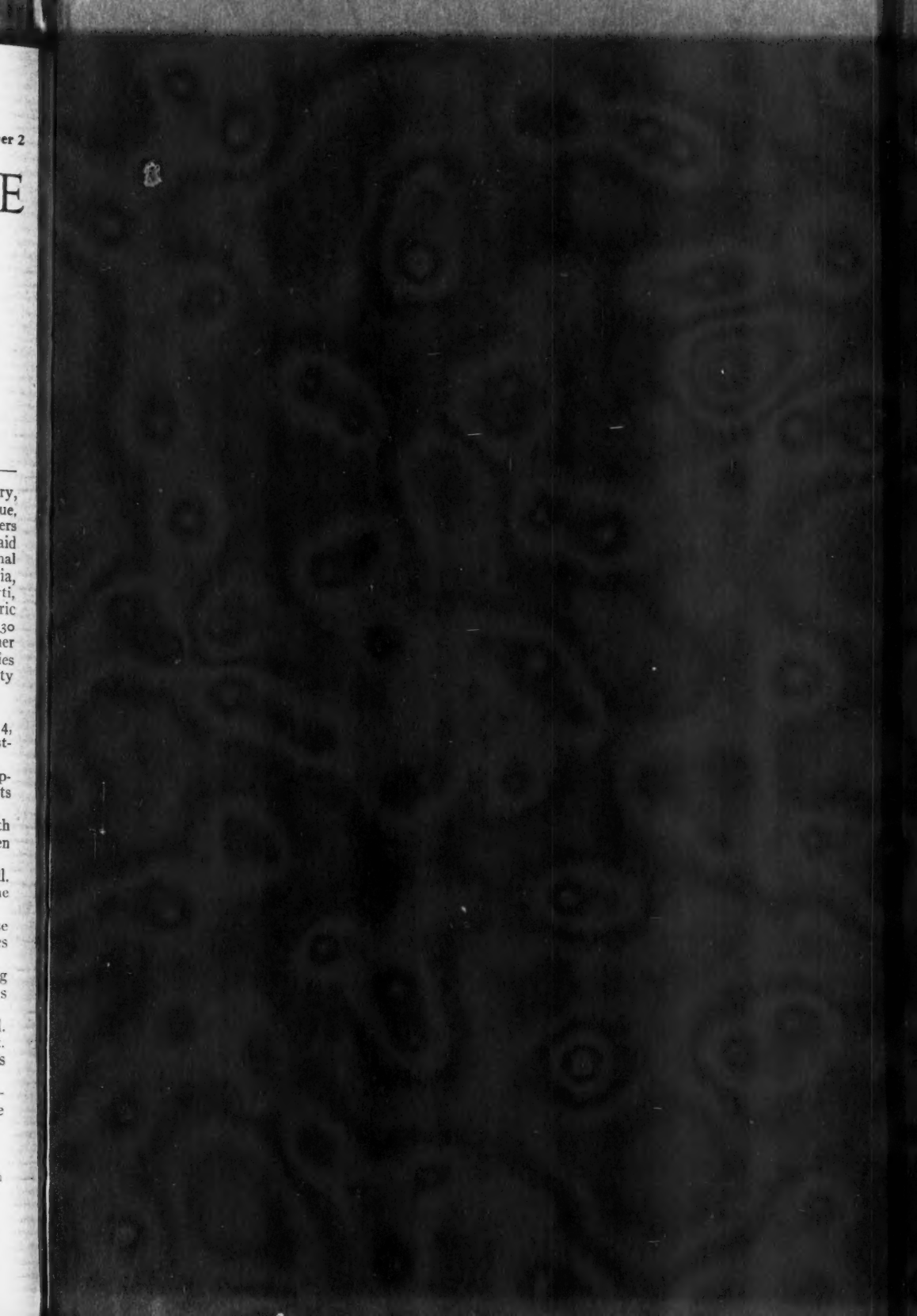

# The PRESS IMPRINT

March and April 1927

## The World and Man

The advance of modern science has revealed a vast store of fascinating facts about the universe in which we live. More discoveries have been made in nearly every branch of science in the past two or three decades than in all the previous history of mankind. Scientists have disclosed a world of the infinitely small in electrons, atoms, and molecules; and of the enormously large in giant suns and galaxies of stars, billions of miles distant. They have looked back millions of years to the origins of worlds, and have traced their history through the geological ages to the present. Myriads of plants and animals have been studied, from the lowliest bacteria and protozoa up to man. Minute investigation of man himself—his origin, the wonders of his body, the development of his intelligence—has yielded astonishing facts. Inanimate matter has revealed marvelous secrets to the modern chemist. The physicist has explained the forces we see about us every day, and the biologist is seeking to explain the very secret of life itself.

To help the inquiring reader in his efforts to keep up with the procession of modern science, sixteen members of the Faculty of the University of Chicago have written *The Nature of the World and of Man*, which E. E. Slosson describes as a book for "those who have not been to college and those who have."

These men, specialists in their fields, are the authors: Forest Ray Moulton, Rollin T. Chamberlin, J Harlen Bretz, Harvey B. Lemon, Julius Stieglitz, Horatio Hackett Newman (the editor), Edwin Oakes Jordan, Merle C. Coulter, Henry Chandler Cowles, Warder C. Allee, Alfred S. Romer, Fay-Cooper Cole, Elliot R. Downing, George W. Bartelmez, Anton J. Carlson, and Charles Hubbard Judd.

Beginning with the outstanding facts about the solar system, each of the sixteen authors has described that phase of development with which he is most familiar: the origin and early stages of the earth; geological processes and the earth's history; the nature of energy and matter; bacteria and their origin; evolution of the plant kingdom, the vertebrates, and the invertebrates; interactions between plants and their environment; the coming of man; organic evolution and the origin of life; human inheritance; structure of man; the living process; and mind in evolution.

Throughout, the authors have emphasized the conception of science as a glowing, living thing inextricably bound up with every human life. With all its multiplicity of authorship, the book has (in the words of the *Chicago Tribune*) "taken on the unity, the coherence, the march of one great epic poem."

Stuart Pratt Sherman said not long ago: "I have a strong impression that our 'average' American is waking up, responding to the new emphasis on 'adult education,' becoming infatuated with the idea of having his education go on as long as he does. He has got wind of the secret that in all the fields of knowledge enormous changes and advances have been made in the last twenty-five years and are still being made at this present hour. His sporting desire to keep up with his times grows keener and the range of his curiosity will soon be encyclopedic."

The popularity of *The Nature of the World and of Man* is evidence of the awakening of the adult public to the possibilities of

further education. For the reader outside the universities the book is a guide to the world of contemporary science; it enables him to form a conception of how the earth came to be, what its place is in the vast physical universe, how life originated; it gives him glimpses of the monsters of early ages; shows him how Cro-Magnon man lived, how atoms are held together in a molecule, how invisible throngs of bacteria surround us, how life came from the sea to the land, why insanity is increasing, how speech developed, how hormones regulate bodily processes. . . . .

*The Nature of the World and of Man* contains the subject matter of a "survey course" given each year by its authors to a selected group of beginning students at the University of Chicago. The success of the experiment upon which it is based suggests a wide use of this text in similar courses in other institutions to give the beginner a preliminary view of the surrounding world and of his possible function in it.

In this day of increasing specialization and a confusingly large body of human knowledge, orientation toward the more general aspects of the world and of man is a necessity. In the belief of the authors of this text, orientation may best begin with a presentation of what modern science holds as truth. They have therefore attempted to give the student that acquaintance with the general outlines of scientific thought which is the ideal point of departure for his entire intellectual development, and which alone affords him the proper perspective on the world of modern thought. More specialized courses in thinking, man in society, or the arts, may well follow, but this course, being more informational, more inclusive, and easier for the beginner should be the point of departure. *THE NATURE OF THE WORLD AND OF MAN*. By SIXTEEN MEMBERS OF THE FACULTY OF THE UNIVERSITY OF CHICAGO. *$4.00, postpaid $4.15.*

* * *

## *Current American Prose*

In a volume of provocative essays, *The Outlook for American Prose*, Joseph Warren Beach searches the current prose of reflection, reminiscence, criticism, and social commentary for auguries of a first-rate style. He judges as stylists the American prose artists who are now in the public eye. Timely and incisive comment from this candid and erudite observer will be not unwelcome to readers who have become aware of the development of American prose style in such books as *Troubadour*, *Straws and Prayer-Books*, and *A Story-Teller's Story*.

A professed eclectic, and adherent of no particular school or tendency, Mr. Beach seeks the perfect style wherever it may be found. Looking about for American writers of cultivated philosophical prose to compare with Bertrand Russell and Havelock Ellis; biographers to rank with Lytton Strachey; newspaper essayists to place beside Chesterton; and critics as stimulating as Shaw, he advances a gratifying number of claimants to distinction. In Sherwood Anderson, James Branch Cabell, H. L. Mencken, Stuart Pratt Sherman, Paul Rosenfeld, Simeon Strunsky, and Alfred Kreymborg, Mr Beach finds the most hopeful signs of a distinguished prose art in America, and whether or not you agree with him, you will have to reckon with his arresting dicta on the luminaries of the day.

"Old fashioned or new fashioned, what we want is something first rate," says Mr. Beach. His regard for stylistic perfection leads him to protest against the incoherence of Hergesheimer; the lack of precision in John Dewey; the naïveté of Dreiser; the scientific jargon of Van Wyck Brooks; and the affectations of Van Vechten. Sentimental violence and overuse of such words as "devastating" and "poignant" are charged against Ben Hecht, Hutchinson, Walpole, and Hergesheimer.

SHERWOOD ANDERSON

—"*A prose-writer who dares to use the devices of the poet.*"

Mr. Beach finds Gamaliel Bradford too serious minded and turns for lightness of touch to Strunsky and Morley. In Anderson, Cabell, Mencken, and Sherman, he finds the supreme qualities of flavor and individuality, gusto, and precision. Kreymborg's style is "uniformly graceful, delicately tinted."

VAN VECHTEN

—"*Baedeker of the intelligentsia.*"

Mr. Beach has put to good use a conception of the genuine critical spirit characterized by candor and tolerance. He admits that he has been denied that arrogance which enables a critic to tell at a glance whether or not a writer is one of the immortals. First-rate prose he finds most frequently in the unpretentious styles that slip in between rigid categories.

You will want to read this book for the vigor of its outlook upon American prose and for its timely comment on some of the most interesting figures in modern literature.

H. L. Mencken says in the *American Mercury:* "This is perhaps the first book, written by an American, in which precept is backed up by practice. Mr. Beach writes very effectively, and has much of interest to say. His judgments on the writing of Dreiser, Anderson, Van Vechten and other living Americans are well-informed and plausible." *THE OUTLOOK FOR AMERICAN PROSE.* By JOSEPH WARREN BEACH. *$2.50, postpaid $2.60.*

* * *

## *New Version of the Psalms*

For a long time we have needed a translation of the Book of Psalms into the language of our day—a translation that would give the modern reader new insight into the classical rendering of the King James Version, and enable him to see in the Psalms the meaning they originally had. Such a translation is the new version by J. M. Powis Smith, which removes the stumbling-blocks of a centuries-old vocabulary, and divests the verses of unwarranted meanings which a succession of early translators read into them. This version is based upon the official Hebrew text, corrected and emended in accordance with the findings of present-day scholarship.

Dr. Smith expresses at the outset his admiration for the King James Version of the Psalms as "a masterpiece of English literature, unrivalled in beauty," and disclaims any attempt to supplant it with his own version. His purpose is rather to express, as completely and as accurately as the limitations of language permit, the thought and feeling of the original; and to incorporate the scholarship of recent decades, with its more thoroughgoing text-critical method and improved philological results. His aim has been at all times to create a clear and uncorrupted version in which the reader of today will be able to see unhampered the beauties and true meaning of the world's greatest hymnbook.

This translation is intended for the public rather than for technical study; it is not, however, an attempt to bring the Psalms down to the level of the mythical "man on the street." The Psalms are here reproduced in a language and style befitting their spirit and content.

While seeking to avoid a bald literalism, Dr. Smith has preferred to err on that side rather than upon the side of paraphrastic expansion. His aim being always accuracy of meaning, he has chosen not to take the liberties of paraphrastic rendering

The translator brings to his task unusual qualifications. He is professor of Semitic languages and literatures in the University of Chicago, editor of the *American Journal of Semitic Languages and Literatures*, and one of the foremost Hebrew scholars in this country. He is the author of several well-known books on Old Testament subjects.

This version of the Psalms is the first step in the complete American translation of the Old Testament, upon which a committee of Hebrew scholars is now working and which will require a year for its completion.

In this rendering, the modern reader can now read the Psalms in the language of his own day, just as they were written centu-

# Teaching Science in the Schools

*By*

ELLIOT R. DOWNING

Scientific study of science teaching has had some valuable results, and these Mr. Downing summarizes in this volume. Here is shown how the growing body of facts about the pedagogy of science, which have been ascertained by the experimental method, may be applied practically to the problems of the science teacher.

This book describes the social and economic background of the science-teaching movement, the history of science teaching in our elementary and secondary schools, present conditions in science teaching, and the aims held.

From this point Mr. Downing takes up the principles of selection and organization of science materials, and actual methods of instruction—by book, lecture-demonstration, and laboratory. There is specific information on notebooks and drawings, field trips, testing results; and suggestions are made for a complete science library in the school.

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That man's brain has been slowly developed from other kinds of animal brain structures, that human behavior patterns and the noblest human faculties are functions of man's peculiar protoplasmic organization—to many these still seem difficult statements to accept. Their truth, however, is further demonstrated by C. Judson Herrick in *Brains of Rats and Men*, his new book on the origin and biological significance of the cerebral cortex. It is a volume that belongs to that rare class of scientific works of genuine literary merit, in which Mr. Herrick traces the evolution of this amazingly complex organ from fishes to men, and follows step by step the elaboration of its functions.

Objective behavior and subjective experience are here reformulated in biological terms. Mr. Herrick demonstrates incisively that the entire intellectual, emotional, and moral life of mankind can be explored as biological functions without loss of their supreme values as distinctively human attributes and without passing outside the realm of natural laws.

Others have tried to read human faculties backward into the lower animals. Mr. Herrick reverses the order and, beginning with the simplest structures prophetic of cerebral cortex, he reads this history through to its culmination in man. He outlines also the development of the thalamus and corpus striatum and their functional relations with the cortex.

He turns then to the mechanism of learning, centering the discussion about the brains of rats and men, the two species of mammals whose behavior patterns have been studied most intensively under conditions of laboratory control. Recent experiments upon learning in rats are summarized.

In considering the transition from brute to man, Mr. Herrick calls attention to the fact that the brain of man is more than twice as heavy as that of an ape of equal

body weight and that this increase is chiefly in the cerebral cortex. The complexity of the internal texture of the human cortex surpasses that of an ape in still larger measure.

This account of the cerebral cortex, its evolution and its functions, by one of America's foremost neurologists touches upon problems that are fundamental to all the sciences that take living things, and man in particular, as their province: biology, psychology, anthropology, the social sciences, education, medicine. H. L. Mencken comments: "It is, perhaps, the best summary of the known facts about mental processes yet put into English." And *The New York World:* "Again and again he discounts with a cool saneness the verbosity of new schools of psychology and philosophy . . . . [it is] suited to any intelligent reader." *BRAINS OF RATS AND MEN.* By C. JUDSON HERRICK. *$3.00, postpaid $3.15.*

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## *Mexico*

Americans give their neighbor a great amount of attention but never, so the Mexicans say, are able to dispose of misconceptions about her. Two of our recent publications, *Some Mexican Problems* by Moises Saenz and Herbert I. Priestley, and *Aspects of Mexican Civilization* by José Vasconcelos and Manuel Gamio, represent the efforts of men who know the country at first hand to outline the true state of affairs at the present time.

Moises Saenz is a member of the Department of Education of Mexico. He gives an authoritative interpretation of the situation created by foreign, particularly American, investments in Mexico; discusses the Mexican laborer; and the progress in integrating Mexico through education. His outline of the program of the Mexican government will tend to correct the impression that the country is given over exclusively to revolutions and political intrigue.

Professor Priestley, of the University of California, author of *The Mexican Nation, a History*, is well qualified to discuss the cultural relations of Mexico and the United States, and the problems which have grown out of Mexico's efforts at rehabilitation. He shows the need for a general reconstruction of Mexican society and ex-


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*By*

HORATIO HACKETT NEWMAN

Attendance at the Dayton trial as a witness for the defense has enabled Professor Newman to describe and interpret it in this new revised edition of his text. He brings the knowledge of the evolutionary biologist to bear upon the present anti-evolution campaign in the United States, and studies it historically, giving the facts about evolution needed for an intelligent attitude toward the controversies.

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## Brains of Rats and Men

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Dr. Herrick traces the evolution of this amazingly complex organ from fishes to men and follows step by step the elaboration of its functions. He demonstrates incisively that the entire intellectual, emotional, and moral life of mankind can be explored as biological functions without passing outside the realm of natural laws.

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amines the working policies and constructive acts of the Mexican people and government. His account is neither a general apologia for the Mexican program nor a wholesale condemnation of it.

Mr. Vasconcelos and Mr. Gamio, both of the Department of Education of Mexico, have outlined Mexican civilization in both its Latin-American and Indian aspects. Mexico is "a country of caste and opposition of every sort, a country of worry and wonder . . . . the rhythm of its very life is the rhythm of contrast," says Mr. Vasconcelos. His essay is an interesting study in contrast.

Mr. Gamio discusses one of Mexico's greatest problems, that of incorporating the Indian in Mexican population. And he has outlined, in the interests of mutual understanding, the efforts of Mexico and the United States to educate one another. *SOME MEXICAN PROBLEMS.* By MOISES SAENZ AND HERBERT I. PRIESTLEY. *ASPECTS OF MEXICAN CIVILIZATION.* By JOSÉ VASCONCELOS AND MANUEL GAMIO. *Each $2.00, postpaid $2.10.*

* * *

## *George Eliot to James Joyce*

"The novels of George Eliot," says Walter L. Myers in *The Later Realism*, "may be named as the first that are adequately representative of native British realism fully matured but not yet modified by French naturalism and later European influences." He traces the development in realistic characterization from George Eliot to the most recent writers of the realistic school.

The writers considered fall into three groups: first, Eliot and Meredith; then, with Hardy as a connecting link, follow Wells, Bennett, and Galsworthy; and finally, Dorothy Richardson, James Joyce, D. H. Lawrence, and May Sinclair.

Mr. Myers has pointed out that the more advanced realistic characterization permits an effect that may properly be called incongruous; that there has been an infusion of something mystical; and that closer attention is being given to personality.

The reader who wishes to keep up with recent developments in British fiction will find this book interesting. *THE LATER REALISM.* By WALTER L. MYERS. *$2.00, postpaid $2.10.*

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VOLUME LXXXIII NUMBER 2

THE

BOTANICAL GAZETTE

*April 1927*

# VIRULENCE, SEROLOGICAL, AND OTHER PHYSIOLOGICAL STUDIES OF BACTERIUM FLACCUMFACIENS, BACT. PHASEOLI, AND BACT. PHASEOLI SOJENSE[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 364

C. G. SHARP

(WITH PLATE VII AND EIGHT FIGURES)

## Introduction

It is a moot question among phytopathologists whether the organisms causing wilt of bean, *Bact. flaccumfaciens*, blight of bean, *Bact. phaseoli*, and pustule of soy bean, *Bact. phaseoli sojense*, deserve species rank or should be considered subspecies or varieties. *Bact. phaseoli* was described by SMITH (**24**) in 1897. He (**25**) describes the symptoms of the disease caused by this organism as follows, stressing the localized lesions in the leaf and stem parenchyma:

> This is a disease of beans common on leaves, stems and pods, and confined principally to the parenchyma although the vessels also are involved, sometimes for a distance of several inches.

BURKHOLDER (**2**) studied bacterial blight of beans, which he describes as a systemic disease caused by *Bact. phaesoli*. He discussed two sets of symptoms, one characterized by lesions on the leaves, stems, and pods as described by SMITH, and commonly known as blight; and a second, unusual systemic symptom, characterized by the presence of the pathogene in the vascular bundles.

[1] Contribution from the department of botany and the department of hygiene and bacteriology, the University of Chicago.

Miss Hedges (9) described a new organism which she had isolated from diseased beans. When inoculated into beans by pricks this organism produced wilt; it also differed from *Bact. phaseoli* culturally, and consequently she named it *Bact. flaccumfaciens*. Another paper by Miss Hedges (13) on a comparative study of *Bact. flaccumfaciens* and *Bact. phaseoli* reported physiological differences in culture media, and differences in staining reaction, and pointed out that *Bact. flaccumfaciens* is a vascular invader, while *Bact. phaseoli* seldom is.

The organism *Bact. phaseoli sojense*, which causes bacterial pustule of soy bean, was described by Miss Hedges (10) in 1922. In 1924 she (11) published a paper on the comparative study of *Bact. phaseoli* and *Bact. phaseoli sojense*, in which she reports that these organisms are different in their pathogenicity for plants (garden bean and soy bean), but that in culture media they are identical. She did, however, in some instances find a morphological difference in the kind of colonies produced, some colonies of *Bact. phaseoli sojense* on thinly sown plates having internal convolutions which were not found in colonies of *Bact. phaseoli*. Wolf (26, 27) concluded that *Bact. phaseoli sojense* is morphologically and culturally indistinguishable from *Bact. phaseoli*. It appears, therefore, that *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense* are very closely related and are not well differentiated, *Bact. flaccumfaciens* standing most apart. The present investigation was undertaken to determine whether these organisms can be differentiated serologically. In the course of the investigation other problems arose, which necessitated a study of (1) the morphology of colonies and of some of the organisms; (2) the physiology of the organisms, specifically their action on various culture media such as milk, starch, gelatin, and sugars; (3) the virulence of the organisms; and (4) the phenomenon of acid agglutination of the organisms.

The cultures used in this study were very kindly furnished by Miss Hedges, U.S. Department of Agriculture, Washington, D.C.

## I. Morphological

Agar shake dilutions were made and plates poured to ascertain whether the cultures were free from contamination and to obtain

single colonies for study. From each culture were isolated two subcultures which were designated as *Bact. flaccumfaciens* no. 7 and no. 8, *Bact. phaseoli* no. 1 and no. 2, and *Bact. phaseoli sojense* smooth (*S*) and rough (*R*) respectively. The last two strains proved to be exceedingly interesting, especially in view of the fact that they came from an agar slant culture which macroscopically appeared smooth.

## Description of colonies

*Bact. flaccumfaciens* no. 7 produced very small round, raised colonies, which were rather dry in consistency, and which did not spread over the medium. *Bact. flaccumfaciens* no. 8 produced very thin flat colonies which were wet and sticky in consistency, and which had a tendency to spread over the medium. On agar slants no. 8 always spread over the surface in a very thin mucoid fashion, most of it settling to the bottom of the slant; no. 7 always remained dry in appearance. Because of these morphological differences, both of these strains were carried through all experiments.

*Bact. phaseoli* no. 1 was isolated from a colony characterized by a hyaline border and concentric rings (figs. 9, 10). On agar slants this culture appeared smoother than no. 2, and had a tendency to settle to the bottom of the slant. *Bact. phaseoli* no. 2 came from a regular raised colony without a hyaline border and without concentric rings (fig. 11).

*Bact. phaseoli sojense* (*S*) came from a round, regular, smooth colony which was very flat and which had a tendency to spread (fig. 12). *Bact. phaseoli sojense* (*R*) came from a colony which was raised and round, and which became umbonate and wrinkled or convoluted in 5–8 days. These colonies were very uneven on the surface (figs. 13–15). A histological study of cross-sections of the rough colony indicates that the irregularities and wrinkles are on the surface of the colony and not within it, as described by Miss Hedges (**11**) for the aberrant colonies noted by her. This phase of the problem is under investigation at present. The colonies of the rough strain, instead of spreading over the medium as does the smooth, grow vertically. Agar slants made from the *R* culture were always rough and much more raised than those from the *S* strain. Both the *S* and *R* strains remained constant on culture media throughout the many

transfers made; however, when a pure line of the *R* strain was inoculated into soy bean plants, both *S* and *R* strains were obtained upon reisolations from the lesions. No change of the *S* to the *R* strain was ever obtained when the former was inoculated into plants. The *S* strain was found to be motile while the *R* strain is non-motile. These observations agree with the findings relative to *S* and *R* forms of certain animal pathogens.

*Bact. flaccumfaciens* no. 7 and no. 8 were both gram positive, while all the other organisms studied were gram negative.

Inoculation experiments proved that each of the subcultures of *Bact. flaccumfaciens* and *Bact. phaseoli* was pathogenic to the garden bean, and that *Bact. phaseoli sojense S* and *R* were pathogenic for the soy bean.

## II. Physiological

Culture media consisting of litmus milk, 0.2 per cent starch agar, 2 per cent plain gelatin, and various sugar broths were used in these studies.

LITMUS MILK.—*Bact. flaccumfaciens* no. 7 and no. 8 both formed acid in 4 days, and caused a reduction of litmus in 25 days. The color of the litmus could be restored by alkali. No coagulation took place in 25 days, but the milk was almost completely peptonized. Neither *Bact. phaseoli* no. 1 and no. 2, nor *Bact. phaseoli sojense S* and *R* produced acid or reduced the litmus in 25 days. More rapid digestion of the milk was produced by the *S* and *R* strains, but all four completely digested milk in 25 days.

STARCH AGAR 0.2 per cent.—Both strains of *Bact. flaccumfaciens* hydrolyzed starch very slowly (fig. 16), while *Bact. phaseoli* no. 1 and no. 2 and *Bact. phaseoli sojense S* and *R* hydrolyzed it rapidly and equally (fig. 17).

PLAIN GELATIN 2 per cent.—The method used here in studying liquefaction of gelatin was that described by LEVINE and CARPENTER (**18, 19**). Twenty gm. of gelatin was added to 1000 cc. of water at 40° C. The material was then incubated at 41° C. for 4 hours, after which the reaction was adjusted to $P_H$ 7.2. Twelve cc. of the medium was now placed in each of a series of test tubes and sterilized in the autoclave.

To compare quantitatively the amount of liquefaction produced in gelatin by *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense* the following experiment was performed. Gelatin cultures were made and permitted to grow for 2 days at 26° C., the gelatin remaining liquid at this temperature. At the end of this period 4 cc. of each of the living cultures was removed aseptically from each tube, 0.5 cc. of which was inoculated into each of eight gelatin tubes. To the remainder of each of the old gelatin cultures was added 1 cc. of chloroform. These were allowed to stand for 4 hours, after which 1 cc. of each of these killed cultures was added to each of eight gelatin tubes. Formol titrations were next made on the 2nd, 5th, 10th, and 25th days. This was done by placing 5 cc. of the gelatin culture in an evaporating dish, adding 20 cc. distilled water, and neutralizing to phenolphthalein; 10 cc. of 50 per cent formalin (neutral to phenolphthalein) was then added, the mixture allowed to stand for 10 minutes, after which it was titrated with N/50 NaOH. The findings are recorded in table I. The results are essentially in accord with those obtained by LEVINE and CARPENTER (**18, 19**), in their work on living and phenolated cultures, and by KENDALL and his co-workers (**17**), on studies with *Proteus*. These investigators state that the slight liquefaction in phenolated tubes (since no growth in them was ever observed) must have been due to the enzymes secreted by the organisms. The conclusion from the data presented in table I is that *Bact. phaseoli* and *Bact. phaseoli sojense* are both rapid liquefiers of gelatin, and cannot be differentiated by this method. On the other hand, *Bact. flaccumfaciens* liquefies gelatin very slowly, and can therefore be differentiated from the other two by this method.

SUGARS.—Six sugars (dextrose, galactose, levulose, lactose, maltose, and sucrose) were used. Tubes containing 8 cc. of standard beef extract-peptone broth, $P_H$ 7.2, were prepared and sterilized in the autoclave. Next 20 per cent solutions of each of the sugars used were prepared and sterilized by filtration; 2 cc. of each was then transferred aseptically to a large number of the tubes containing plain broth, thus making a 4 per cent sugar broth. These sugar media were allowed to incubate for 4 days before use to prove sterility. Control tubes (uninoculated) were carried throughout the experiment.

In each experiment duplicate and often triplicate tests were made and the average $P_H$ for these taken. This was determined colorimetrically, using different indicators to check the accuracy of the

TABLE I

EFFECT OF PRESENCE OF ORGANISMS STUDIED ON FORMOL TITRATION OF GELATIN WHEN CHLOROFORMED AND NOT CHLOROFORMED, AND ON FINAL $P_H$

| Organism | Days incubated at 30° C. | cc. N/1 NaOH per 100 cc. gelatin | | | | | | Gelatin | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Not chloroformed | | | Chloroformed | | | | | |
| | | Control gelatin | Organism added | Increase over control | Control gelatin | Organism added | Increase over control | Control ($P_H$) | Not chloroformed ($P_H$) | Chloroformed ($P_H$) |
| Bact. phaseoli no. 1 | 2 | 0.56 | 0.80 | 0.30 | 0.56 | 0.64 | 0.08 | 6.6 | 7.8 | 6.4 |
| | 5 | 0.40 | 0.96 | 0.56 | 0.40 | 0.48 | 0.08 | | | |
| | 10 | 0.56 | 2.28 | 1.72 | 0.56 | 0.68 | 0.12 | | | |
| | 25 | 0.60 | 8.84 | 8.24 | 0.60 | 0.96 | 0.36 | | | |
| Bact. phaseoli no. 2 | 2 | 0.56 | 0.96 | 0.40 | 0.56 | 0.72 | 0.16 | 6.6 | 7.6 | 6.5 |
| | 5 | 0.40 | 1.72 | 1.32 | 0.40 | 0.64 | 0.24 | | | |
| | 10 | 0.56 | 4.04 | 3.48 | 0.56 | 1.04 | 0.48 | | | |
| | 25 | 0.60 | 9.72 | 9.12 | 0.60 | 1.64 | 1.04 | | | |
| Bact. phaseoli sojense *S* | 2 | 0.56 | 0.68 | 0.12 | 0.56 | 0.64 | 0.08 | 6.6 | 7.6 | 6.5 |
| | 5 | 0.40 | 1.08 | 0.68 | 0.40 | 0.52 | 0.12 | | | |
| | 10 | 0.56 | 4.08 | 3.52 | 0.56 | 0.72 | 0.16 | | | |
| | 25 | 0.60 | 9.92 | 9.32 | 0.60 | 1.04 | 0.44 | | | |
| Bact. phaseoli sojense *R* | 2 | 0.56 | 0.76 | 0.20 | 0.56 | 0.64 | 0.08 | 6.6 | 7.6 | 6.4 |
| | 5 | 0.40 | 0.96 | 0.56 | 0.40 | 0.52 | 0.12 | | | |
| | 10 | 0.56 | 3.80 | 2.24 | 0.56 | 0.76 | 0.20 | | | |
| | 25 | 0.60 | 9.44 | 8.84 | 0.60 | 1.04 | 0.44 | | | |
| Bact. flaccumfaciens no. 7 | 2 | 0.56 | 0.56 | 0.00 | 0.56 | 0.56 | 0.00 | 6.6 | 7.0 | 6.5 |
| | 5 | 0.40 | 0.40 | 0.00 | 0.40 | 0.40 | 0.00 | | | |
| | 10 | 0.56 | 0.72 | 0.16 | 0.56 | 0.56 | 0.00 | | | |
| | 25 | 0.60 | 1.64 | 1.04 | 0.60 | 0.60 | 0.00 | | | |
| Bact. flaccumfaciens no. 8 | 2 | 0.56 | 0.58 | 0.02 | 0.56 | 0.58 | 0.02 | 6.6 | 6.8 | 6.5 |
| | 5 | 0.40 | 0.40 | 0.00 | 0.40 | 0.40 | 0.00 | | | |
| | 10 | 0.56 | 0.72 | 0.16 | 0.56 | 0.58 | 0.02 | | | |
| | 25 | 0.60 | 0.84 | 0.24 | 0.60 | 0.60 | 0.00 | | | |

work. The results of this experiment are recorded in table II. From these data it is seen that *Bact. flaccumfaciens* produced acid in all the sugars used, and thus can be differentiated from the other two organisms. *Bact. phaseoli* and *Bact. phaseoli sojense* produced little

## TABLE II

CHANGE IN H-ION CONCENTRATION OF VARIOUS SUGAR MEDIA BY BACT. FLACCUMFACIENS, BACT. PHASEOLI, AND BACT. PHASEOLI SOJENSE

| ORGANISM | DEXTROSE $P_H$ 7.2* | | | | | GALACTOSE $P_H$ 7.1* | | | | | LEVULOSE $P_H$ 6.9* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $P_H$ after no. of days | | | | | $P_H$ after no. of days | | | | | $P_H$ after no. of days | | | | |
| | 5 | 10 | 20 | 30 | 40 | 5 | 10 | 20 | 30 | 40 | 5 | 10 | 20 | 30 | 40 |
| Control | 7.2 | 7.2 | 7.1 | 7.1 | 7.1 | 7.1 | 7.1 | 7.1 | 7.1 | 7.1 | 6.9 | 6.8 | 6.8 | 6.8 | 6.8 |
| Bact. phaseoli no. 1 | 7.2 | 7.4 | 7.3 | 7.0 | 6.8 | 7.1 | 7.2 | 6.7 | 6.6 | 6.0 | 7.1 | 6.8 | 6.6 | 6.2 | 7.2 |
| Bact. phaseoli no. 2 | 7.3 | 7.4 | 7.3 | 7.3 | ..... | 7.2 | 7.1 | 6.8 | 6.8 | 6.4 | 7.1 | 7.2 | 6.8 | 6.8 | 7.0 |
| Bact. phaseoli sojense *S*. | 7.3 | 7.4 | 7.4 | 7.5 | ..... | 7.2 | 7.1 | 7.0 | 6.6 | ..... | 7.2 | 7.3 | 7.3 | 7.3 | 7.4 |
| Bact. phaseoli sojense *R*. | 7.3 | 7.4 | 7.3 | 7.2 | 6.9 | 7.2 | 7.0 | 6.8 | 6.4 | 6.0 | 7.2 | 7.4 | 7.4 | 7.4 | 7.4 |
| Bact. flaccumfaciens 7 | 7.0 | 6.0 | 5.2 | 4.6 | 4.6 | 7.0 | 6.6 | 5.2 | 4.8 | 4.8 | 6.3 | 5.2 | 5.0 | 4.8 | 4.8 |
| Bact. flaccumfaciens 8 | 7.0 | 6.8 | 5.7 | 4.6 | 4.6 | 7.1 | 6.7 | 6.3 | 5.2 | 5.2 | 6.2 | 5.3 | 5.1 | 4.8 | 4.8 |
| | LACTOSE $P_H$ 7.1* | | | | | MALTOSE $P_H$ 7.1* | | | | | SUCROSE $P_H$ 7.3* | | | | |
| Control | 7.1 | 7.1 | 7.1 | 7.1 | 7.1 | 7.1 | 7.2 | 7.1 | 7.1 | 7.1 | 7.3 | 7.3 | 7.3 | 7.2 | 7.2 |
| Bact. phaseoli no. 1 | 7.4 | 7.4 | 7.5 | 7.6 | 7.6 | 7.2 | 7.2 | 7.1 | 7.0 | 6.8 | 7.4 | 7.2 | 7.0 | 6.8 | 7.0 |
| Bact. phaseoli no. 2 | 7.3 | 7.2 | 7.2 | 7.1 | 6.6 | 7.2 | 7.2 | 7.2 | 7.1 | 7.1 | 7.4 | 7.3 | 7.2 | 7.1 | 6.6 |
| Bact. phaseoli sojense *S*. | 7.6 | 7.7 | 7.9 | 8.2 | 8.2 | 7.4 | 7.4 | 7.2 | 7.3 | 7.3 | 7.4 | 7.4 | 7.4 | 7.3 | 7.3 |
| Bact. phaseoli sojense *R*. | 7.6 | 7.7 | 7.9 | 8.2 | 8.2 | 7.4 | 7.2 | 7.2 | 7.1 | 7.4 | 7.4 | 7.4 | 7.4 | 7.1 | 6.6 |
| Bact. flaccumfaciens 7 | 7.1 | 6.9 | 6.6 | 5.7 | 5.2 | 7.2 | 6.7 | 5.6 | 4.6 | 4.6 | 7.1 | 6.4 | 5.4 | 4.8 | 4.6 |
| Bact. flaccumfaciens 8 | 7.1 | 6.9 | 6.2 | 5.7 | 5.5 | 7.2 | 6.8 | 5.8 | 5.5 | 5.2 | 7.2 | 6.8 | 5.8 | 4.9 | 4.8 |

* Original $P_H$ of media.

change in 30 days in dextrose, maltose, and sucrose, but formed acid in galactose. *Bact. phaseoli* in 30 days changed levulose from $P_H$ 6.8 to 6.2–6.8, and lactose from $P_H$ 7.1 to 7.6; while *Bact. phaseoli sojense* changed levulose to $P_H$ 7.3 and lactose to $P_H$ 8.2. *Bact. phaseoli sojense S* produces a very scaly pellicle which is very characteristic on lactose and levulose, and resembles duckweed on a pond. *B. phaseoli sojense R* in most of these sugars produced a very heavy granular precipitate.

The conclusion drawn from these data is that since *Bact. phaseoli* no. 1 and no. 2 did not always act alike, producing at times different quantities of acid and alkali in various sugars, it would be unwise to assert that the differences obtained between *Bact. phaseoli* and *Bact. phaseoli sojense* are sufficient to differentiate them. It is possible that other strains of each of these organisms might act exactly alike on these sugars. The alkalinity produced in levulose and lactose by *Bact. phaseoli* and *Bact. phaseoli sojense* is probably due to their hydrolytic action on the proteins in the broth yielding products with alkaline reactions, and not to any change occurring in the sugars.

## III. Serological

### A. Agglutinin test

For the serological differentiation of *Bact. flaccumfaciens* no. 7 and no. 8, *Bact. phaseoli* no. 1 and no. 2, and *Bact. phaseoli sojense S* and *R* the agglutination and precipitation tests were used. The agglutinin test, because of its partial specificity, has become one of the most important methods for the diagnosis of various animal diseases and for the classification of animal pathogenes. Heretofore little use has been made of it in the field of plant pathology for the diagnosis of disease or identification of pathogenes. The literature for this subject is reviewed in the accompanying paper by Link and Sharp.

Method of procedure.—After all the subcultures under consideration had been proved pathogenic to bean plants, each culture was grown on standard potato dextrose agar slants ($P_H$ 7.2) for 4 days. The slants were then washed off with 0.85 per cent salt solution, and an even suspension of each organism was made to contain

## TABLE III

AGGLUTINATION TESTS USING BACT. FLACCUMFACIENS NO. 7 ANTISERUM AGAINST SUSPENSIONS

| DILUTION OF SERUM | BACT. FLACCUMFACIENS NO. 7 | | | BACT. FLACCUMFACIENS NO. 8 | | | BACT. PHASEOLI NO. 1 | | BACT. PHASEOLI NO. 2 | | BACT. PHASEOLI SOJENSE S | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tests | | | Tests | | | Tests | | Tests | | Tests | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 1 | 2 | 1 | 2 |
| 1:5 | + | + | +++* | − | − | +++ | − | − | − | − | − | − |
| 1:10 | ++ | ++ | +++ | − | − | ++++* | − | − | − | − | − | − |
| 1:20 | ++* | +++ | ++++ | − | + | ++++* | − | − | − | − | − | − |
| 1:40 | ++* | ++++ | +++ | − | + | ++++* | − | − | − | − | − | − |
| 1:80 | ++++† | ++++ | ++ | − | + | ++ | − | − | − | − | − | − |
| 1:160 | +++ | ++ | +* | − | + | + | − | − | − | − | − | − |
| 1:320 | ++ | + | − | − | − | − | − | − | − | − | − | − |
| 1:640 | + | − | − | − | − | − | − | − | − | − | − | − |
| 1:1280 | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:2560 | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:5120 | − | − | − | − | − | − | − | − | − | − | − | − |
| Saline | − | − | − | − | − | − | − | − | − | − | − | − |

* Slightly less than the number of pluses indicated.

† In this and all the following tables where the symbols are used, ++++ stands for complete agglutination, and +++, ++, and + stand respectively for three-fourths, one-half, and one-fourth complete agglutination.

## TABLE IV

AGGLUTINATION TESTS USING BACT. PHASEOLI NO. 1 ANTISERUM AGAINST SUSPENSIONS

| DILUTION OF SERUM | BACT. PHASEOLI NO. 1 | | | BACT. PHASEOLI NO. 2 | | | BACT. PHASEOLI SOJENSE S | | | BACT. FLACCUMFACIENS NO. 7 | | BACT. FLACCUMFACIENS NO. 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tests | | | Tests | | | Tests | | | Tests | | Tests | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 1 | 2 |
| 1:5 | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++* | ++ | + | + | + | + | + |
| 1:10 | ++++ | +++ | ++++ | ++++ | ++++ | ++++ | + | ++* | + | − | − | − | − |
| 1:20 | ++++ | +++ | +++ | ++++ | ++++* | ++++* | + | + | − | − | − | − | − |
| 1:40 | ++++ | ++ | ++ | ++++ | +++ | +++ | + | + | − | − | − | − | − |
| 1:80 | ++++ | ++* | ++* | ++++ | +++ | ++ | + | + | − | − | − | − | − |
| 1:160 | +++ | + | + | +++ | ++ | + | − | − | − | − | − | − | − |
| 1:320 | ++* | − | − | ++ | − | − | − | − | − | − | − | − | − |
| 1:640 | + | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:1280 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:2560 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:5120 | − | − | − | − | − | − | − | − | − | − | − | − | − |

* Slightly less than the number of pluses indicated.

## TABLE V

AGGLUTINATION TESTS USING BACT. PHASEOLI SOJENSE *S* ANTISERUM AGAINST SUSPENSIONS

| DILUTION OF SERUM | BACT. PHASEOLI SOJENSE *S* | | | BACT. PHASEOLI NO. 1 | | | BACT. PHASEOLI NO. 2 | | | BACT. FLACCUMFACIENS NO. 7 | | BACT. FLACCUMFACIENS NO. 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tests | | | Tests | | | Tests | | | Tests | | Tests | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 1 | 2 |
| 1:5 | ++++ | ++++ | ++++ | ++* | ++ | + | ++* | + | + | + | ++* | + | ++* |
| 1:10 | ++++ | ++++ | +++ | + | + | − | + | − | − | − | − | − | + |
| 1:20 | ++++ | ++++ | + | − | − | − | − | − | − | − | − | − | − |
| 1:40 | ++++ | ++ | − | − | − | − | − | − | − | − | − | − | − |
| 1:80 | ++++ | + | − | − | − | − | − | − | − | − | − | − | − |
| 1:160 | ++++ | + | − | − | − | − | − | − | − | − | − | − | − |
| 1:320 | ++++ | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:640 | +++ | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:1280 | + | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:2560 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:5120 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Saline | − | − | − | − | − | − | − | − | − | − | − | − | − |

* Slightly less than the number of pluses indicated.

approximately two billion organisms per cc. Next the suspensions were heated for 1 hour at 60° C. to kill the organisms. These suspensions were then used as the stock antigen.

Five intraperitoneal injections were made into rabbits at 4-day intervals, using 50 million dead organisms for the first injection, 100 million for the second, and 200 million for each of the last three injections. Five days after the last injection the rabbits were bled and the serum prepared. Agglutination tests were now set up, using

TABLE VI

AGGLUTINATION TESTS USING NORMAL SERUM AS CONTROL AGAINST SUSPENSIONS

| DILUTION OF SERUM | BACT. PHASEOLI SOJENSE *S* | | | BACT. PHASEOLI NO. 1 | | | BACT. PHASEOLI NO. 2 | | | BACT. FLACCUMFACIENS NO. 7 | | BACT. FLACCUMFACIENS NO. 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tests | | | Tests | | | Tests | | | Tests | | Tests | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 1 | 2 |
| 1:5 | − | − | − | − | − | − | − | − | − | + | + | + | + |
| 1:10 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:20 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:40 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:80 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:160 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:320 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:640 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:1280 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:2560 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 1:5120 | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Saline | − | − | − | − | − | − | − | − | − | − | − | − | − |

the serum prepared from each organism with its homologous antigen as well as with the other antigens, but no agglutination was obtained. This may mean that when they were killed by heat: (1) the antigenic properties of the organisms were destroyed; (2) the doses used were too small or too few; or (3) both of these factors were involved.

Heavy suspensions of the living organisms were next prepared, and four intravenous injections were made into the ear of each of the rabbits at intervals of 4 days, using 0.5, 0.75, 1, and 1 cc. respectively. Twelve days after the last injection the rabbits were again bled, serum prepared, and agglutination tests set up.[2]

[2] For method of setting up the tests, and a typical protocol, see accompanying paper by LINK and SHARP.

The organisms used for the suspensions in the agglutination tests were grown on standard beef extract-peptone-dextrose agar. The bacteria were washed off in 0.85 per cent salt solution, washed twice by centrifuging, and were re-suspended after the second washing in salt solution, the last suspension being used for the agglutination tests. The organisms were grown on different media and were washed

FIG. 1.—Bean plants 21 days after inoculation with *B. flaccumfaciens* no. 7; only slight infection present.

in order to prevent any possible chance of a precipitin reaction with the protein from the potato agar used for preparing the antigen.

The protocols of the experiments are given in tables III–VI. Table III shows that the antiserum of *Bact. flaccumfaciens* no. 7 gives specific agglutination with its own homologous strain and with no. 8, but that it fails entirely to agglutinate *Bact. phaseoli* no. 1 or no. 2 and *Bact. phaseoli sojense S.* No explanation can be given for not obtaining agglutination in test 1 against *Bact. flaccumfaciens* no. 8.

The results in test 1 (tables III–V) were obtained with serum

prepared from the second bleeding of the rabbits 12 days after the last injection. Since there was not enough serum left to recheck the experiment, a third bleeding was made 10 days after the second bleeding, and tests 2 and 3 made with the last lot of serum obtained. No injection of antigen was made between the second and third bleeding.

FIG. 2.—Same bean plants 21 days after inoculation with *B. flaccumfaciens* no. 8; plant not inoculated not infected, others nearly dead.

In all the tables the serum used in test 1 showed a higher titre than that used for tests 2 and 3. This indicates that the bleeding of the rabbits or the time elapsing after the last injection of antigen caused a reduction in the titre of all the sera used. Another very interesting phenomenon noted in all the tests reported in table III was that of the zone phenomenon. In dilution of 1–5 little agglutination occurred, while in dilutions 1–40 to 1–80 of tests 1 and 2, and 1–20 to 1–40 of test 3 there was almost complete agglutination in every case. Antiserum from strain no. 8 of *Bact. flaccumfaciens* was also used,

and it gave practically the same results as that obtained with antiserum from strain no. 7; therefore the conclusions are that antiserum from *Bact. flaccumfaciens* no. 7 and no. 8 will not serve to differentiate these two strains, but will differentiate *Bact. flaccumfaciens* from *Bact. phaseoli* and from *Bact. phaseoli sojense S.*

The data in table IV show that the antiserum from *Bact. phaseoli* no. 1 will differentiate the two strains no. 1 and no. 2 from *Bact.*

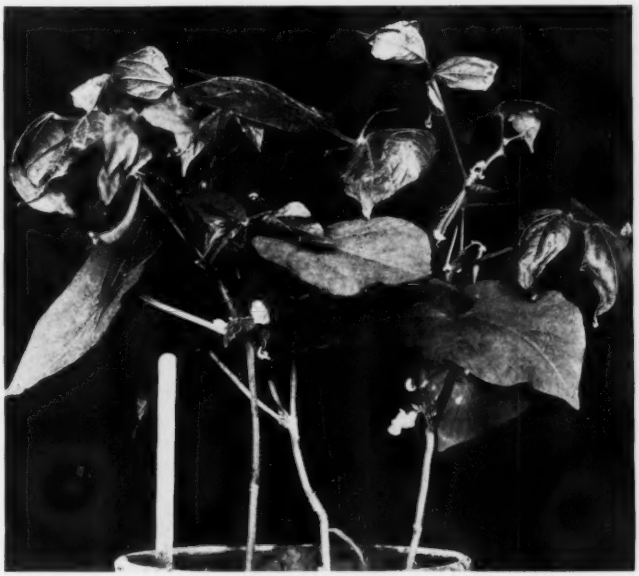

FIG. 3.—Bean plants 21 days after inoculation with *B. phaseoli* no. 1; extensive lesions present.

*phaseoli sojense S,* and *Bact. flaccumfaciens* no. 7 and no. 8. It also shows a slight group agglutination with *Bact. phaseoli sojense S* which may mean, according to current serological interpretation, that *Bact. phaseoli* contains a protein capable of stimulating slight antibody production against *Bact. phaseoli sojense S.*

The antiserum from *Bact. phaseoli* no. 2 acted in practically the same manner as the antiserum from no. 1, except that it failed to give quite as much group agglutination with *Bact. phaseoli sojense S;*

hence the two strains of *Bact. phaseoli* are identical serologically, but differ from the other two organisms. Practically no agglutination was obtained with *B. flaccumfaciens* no. 7 and no. 8, which indicates that, serologically at least, *Bact. phaseoli* and *B. phaseoli sojense* are more closely related than they are to *B. flaccumfaciens*. This is in harmony with the results obtained in the physiological studies reported, in which it was shown that *Bact. phaseoli* and *Bact. phaseoli*

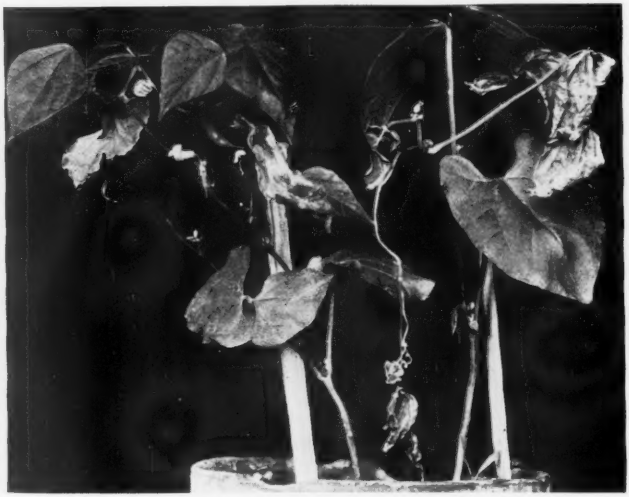

FIG. 4.—Bean plants 21 days after inoculation with *B. phaseoli* no. 2; top completely dead in one plant.

*sojense* are similar but distinct from *B. flaccumfaciens* in their action upon starch agar, gelatin, and sugars.

Table V shows that the antiserum from *Bact. phaseoli sojense S* is highly specific for its homologous organism, and will differentiate this from the other two organisms. Antiserum prepared from *Bact. phaseoli sojense R* gave the same results as that from the *S* strain; however, no agglutination tests could be run against the *R* strain, since this is spontaneously agglutinated in both distilled water and in salt solution. Since the antisera from both organisms act alike, it is probable that they are chemically the same, and differ only in

their physical properties. Serologically they both differ from *Bact. phaseoli* and *Bact. flaccumfaciens*. Table VI shows that none of the organisms was agglutinated by normal rabbit serum.

## B. Precipitin test

Griffith (**8**), and Jacobson and Falk (**14, 15**) have shown that when *S* and *R* strains of pneumococci are grown in broth, the former produces in 4–16 hours a specific soluble substance which when

TABLE VII

Precipitin test using antiserum from B. phaseoli sojense smooth, against filtered broths from smooth and rough strains of B. phaseoli sojense

| Amount of serum (cc.) | Source of broth | Amount of broth (cc.) | Results |
|---|---|---|---|
| 0.5 | Smooth | 0.5 | ++++ |
| 0.25 | Smooth | 0.75 | +++ |
| 0.125 | Smooth | 0.875 | + |
| 0.5 | Rough | 0.5 | ++++ |
| 0.25 | Rough | 0.75 | ++++ |
| 0.125 | Rough | 0.875 | + |
| 0.5 | (Sterile control) | 0.5 | − |
| 0.25 | (Sterile control) | 0.75 | − |
| 0.125 | (Sterile control | 0.875 | − |

mixed with homologous antiserum will give the precipitin reaction. The *R* strain gives no precipitation early, however, but after 24–48 hours will give a reaction with its antiserum.

Since it was found possible to differentiate *S* and *R* strains of pneumococci in this manner, it was decided to apply this technique to *Bact. phaseoli sojense S* and *R* strains, to see whether it could also be used to differentiate these strains. Table VII gives the protocol of the experiment. Standard beef extract-peptone-dextrose broth was used. The organisms were allowed to grow in separate flasks for 8 days, after which the culture media were passed through Berkefeld filters to obtain a clear solution free from bacteria. When antiserum prepared from either *S* or *R* strains was added to the filtrates in suitable quantities a precipitation occurred in each case, showing

that the test was non-specific for the strains. No precipitation was obtained when sterilized plain broth was used. The test was repeated later but the organisms were allowed to grow only 6 days. No precipitation was obtained. The conclusions are that (1) the first precipitation obtained was probably due to autolytic products of the bacteria

FIG. 5.—Keeney beans 19 days after being inoculated with *B. phaseoli* no. 2

and not to any soluble substance produced; (2) the *S* and *R* strains cannot be differentiated by the precipitin test when filtrates of broth cultures are used.

## IV. Virulence

### BACT. FLACCUMFACIENS AND BACT. PHASEOLI

In studying virulence of the pathogenes under consideration, the following varieties of beans were used: Challenge Dwarf Black Wax, Keeney's Rustless Golden Wax, and Manchu soy bean. *Bact.*

*flaccumfaciens* no. 7 and no. 8, *Bact. phaseoli* no. 1 and no. 2, and *Bact. phaseoli sojense S* and *R* respectively were inoculated into these varieties.

In all experiments on virulence the plants were inoculated by pricking with a needle, by spraying, or by rubbing the leaves and

FIG. 6.—Challenge beans 19 days after being inoculated with *B. phaseoli* no. 2; lesions much more severe than in fig. 5.

stems. After the plants were inoculated they were set in a moist chamber for 48 hours, and then removed to the greenhouse where the temperature ranged from 75°–112° F. In every case the organisms were much more virulent at the higher temperatures.

*Bact. flaccumfaciens* no. 7 when pricked or rubbed into the leaves or stems of the Challenge or Keeney bean produced only slight infec-

tion, only two or three leaves showing wilting or lesions at any one time (fig. 1). This strain of the organism was never reisolated from the plant. Culturally and serologically it was the same as no. 8. *Bact. flaccumfaciens* no. 8 when inoculated into the same varieties by the same method used for no. 7 produced wilting in 9 days, signs of which appeared as early as the fourth day. The plants soon ceased to grow, and at the end of 21 days were almost dead (fig. 2). The non-inoculated plant in the same pot remained healthy, thus confirming statements in the literature that this organism gains entrance only through wounds. No infection was ever produced in the soy bean by either no. 7 or no. 8. Strain no. 8 was reisolated from diseased plants and upon further inoculations again proved pathogenic.

FIG. 7.—Characteristic lesions on soy bean leaf produced by *B. phaseoli sojense;* inoculations made by needle pricks.

*Bact. phaseoli* no. 1 and no. 2 both proved pathogenic to the garden bean when the leaves were either sprayed, rubbed, or pricked with 48-hour old suspensions of the organisms; however, no. 1 was less virulent than no. 2 in every experiment performed (figs. 3, 4). When the plants were inoculated by injecting suspensions of the organisms into the stem no difference in virulence was noted in no. 1 and no. 2. Production of the disease by stem inoculation showed that the organism can invade the vascular system when wounds are present. Yellow exudations of bacteria were formed at the point of inoculation. It was also found that in every instance the Keeney bean was more resistant to both *Bact. phaseoli* and *Bact. flaccumfaciens* than was the Challenge bean (figs. 5, 6). Both no. 1 and no. 2 were reisolated and proved pathogenic upon reinoculation.

### BACT. PHASEOLI SOJENSE *S* AND *R*

When suspensions of the *S* and *R* strains were inoculated into the leaves of soy beans by spraying or rubbing no difference in the

pathogenicity of these strains was observed; however, when the leaves were inoculated by needle pricks a difference in percentage of infection and in size of lesions was noted. In making the needle prick inoculations, the needle was dipped into a suspension of the organism, and five punctures made in the leaf, after which the needle was again placed in the inoculum and five more punctures made. This operation was continued until 150 punctures were made with each of the strains. Following inoculation the plants were kept in a

TABLE VIII

PERCENTAGE OF INFECTION ON DIFFERENT DAYS RESULTING FROM 150 PUNCTURES WITH SUSPENSIONS OF BACT. PHASEOLI SOJENSE SMOOTH AND ROUGH

| EXPERIMENT | PERCENTAGE OF INFECTION | | | REMARKS |
|---|---|---|---|---|
| | 4 days | 5 days | 6 days | |
| 1. Smooth | ..... | 56 | 80 | In 8–10 days there was practically 100 per cent infection from both smooth and rough; lesions from smooth organisms were always larger with larger and much more definite yellow borders than from rough |
| Rough | ..... | 26 | 74 | |
| 2. Smooth | 64 | 91.8 | ..... | |
| Rough | 30 | 88.0 | ..... | |
| 3. Smooth | 71 | ..... | ..... | |
| Rough | 66 | ..... | ..... | |
| 4. Smooth | 39 | ..... | ..... | |
| Rough | 7 | ..... | ..... | |
| 5. Smooth | ..... | 90 | ..... | |
| Rough | ..... | 57 | ..... | |
| 6. Smooth | 75 | ..... | ..... | |
| Rough | 52 | ..... | ..... | |

moist chamber for 48 hours, and then removed to the greenhouse where daily observations were made. In each of the six experiments infection took place more rapidly with the *S* than with the *R* strain (table VIII).

The lesions produced by the *S* strain always appeared larger than those produced by the *R* strain. In order to determine that they really were larger they were accurately measured. The leaves were inoculated by needle pricks (fig. 7), and when the lesions had developed, after 5–8 days, the leaves were removed from the plants. They were next placed between two large pieces of glass which were

bound together by adhesive tape, so that measurements could be made under low power of the microscope by use of an ocular microm-

TABLE IX

SIZE OF LESIONS PRODUCED ON SOY BEANS BY BACT. PHASEOLI SOJENSE SMOOTH AND ROUGH

| | Experiment no. 1 inoculated August 20; measured August 28, 1926 | | | | Experiment no. 2 inoculated August 22; measured August 28, 1926 | | | | Experiment no. 3 inoculated August 25; measured August 30, 1926 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Smooth | | Rough | | Smooth | | Rough | | Smooth | | Rough | |
| | Diameter* | F† | Diameter | F | Diameter | F | Diameter | F | Diameter | F | Diameter | F |
| | 18 | 1 | 14 | 1 | 12 | 1 | 12 | 1 | 12 | 2 | 10 | 2 |
| | 21 | 2 | 18 | 3 | 19 | 1 | 14 | 1 | 15 | 5 | 11 | 2 |
| | 22 | 3 | 20 | 4 | 20 | 4 | 15 | 3 | 16 | 5 | 12 | 5 |
| | 23 | 1 | 21 | 3 | 21 | 1 | 16 | 1 | 17 | 5 | 13 | 4 |
| | 24 | 1 | 22 | 3 | 22 | 1 | 17 | 1 | 18 | 5 | 14 | 9 |
| | 25 | 2 | 23 | 3 | 24 | 2 | 18 | 1 | 19 | 5 | 15 | 9 |
| | 26 | 1 | 24 | 1 | 25 | 1 | 19 | 2 | 20 | 10 | 16 | 11 |
| | 28 | 1 | 25 | 3 | 27 | 2 | 20 | 3 | 21 | 6 | 17 | 7 |
| | 29 | 2 | 26 | 1 | 28 | 3 | 21 | 1 | 22 | 10 | 18 | 8 |
| | 30 | 2 | 27 | 1 | 29 | 1 | 22 | 1 | 23 | 10 | 19 | 4 |
| | 31 | 1 | 28 | 2 | 30 | 6 | 23 | 3 | 24 | 7 | 20 | 8 |
| | 32 | 1 | 29 | 1 | 31 | 1 | 24 | 1 | 25 | 9 | 21 | 8 |
| | 33 | 1 | 30 | 2 | 32 | 3 | 25 | 7 | 26 | 6 | 22 | 6 |
| | 34 | 5 | 31 | 1 | 33 | 5 | 26 | 1 | 27 | 6 | 23 | 8 |
| | 35 | 2 | 32 | 1 | 34 | 1 | 27 | 3 | 28 | 5 | 24 | 2 |
| | 36 | 2 | 33 | 1 | 35 | 4 | 28 | 5 | 29 | 3 | 25 | 4 |
| | 37 | 1 | 34 | 2 | 37 | 2 | 30 | 6 | 30 | 11 | 27 | 3 |
| | 38 | 2 | 35 | 12 | 38 | 2 | 31 | 1 | 32 | 5 | 28 | 4 |
| | 40 | 7 | 36 | 1 | 40 | 2 | 32 | 2 | 33 | 2 | 30 | 4 |
| | 41 | 1 | 37 | 1 | 42 | 1 | 33 | 1 | 34 | 7 | 32 | 2 |
| | 42 | 2 | 38 | 2 | 43 | 1 | 36 | 1 | 36 | 6 | 33 | 2 |
| | 43 | 1 | 39 | 3 | 45 | 3 | 40 | 3 | 37 | 2 | 34 | 1 |
| | 45 | 5 | 40 | 3 | 50 | 2 | 50 | 1 | 40 | 5 | 36 | 2 |
| | 48 | 3 | 41 | 1 | ..... | ..... | ..... | ..... | 44 | 2 | 37 | 1 |
| | 50 | 5 | 42 | 1 | ..... | ..... | ..... | ..... | 45 | 7 | 38 | 1 |
| | 52 | 3 | 43 | 3 | ..... | ..... | ..... | ..... | 50 | 6 | 40 | 1 |
| | 53 | 1 | 44 | 1 | ..... | ..... | ..... | ..... | 52 | 1 | 41 | 1 |
| | 55 | 3 | 45 | 5 | ..... | ..... | ..... | ..... | 55 | 5 | ..... | ..... |
| | 58 | 2 | 46 | 2 | ..... | ..... | ..... | ..... | 57 | 1 | ..... | ..... |
| | 65 | 4 | 52 | 1 | ..... | ..... | ..... | ..... | 60 | 2 | ..... | ..... |
| Total..... | 2734 | 68 | 2238 | 69 | 1590 | 50 | 1292 | 50 | 4654 | 161 | 2427 | 161 |
| Average size... | 40.2 ±0.49 | | 32.43 ±0.36 | | 31.8 ±0.39 | | 25.84 ±0.44 | | 28.92 ±0.30 | | 20.4 ±0.2 | |
| Average size mm..... | 1.61 ±0.02 | | 1.28 ±0.014 | | 1.26 ±0.016 | | 1.03 ±0.018 | | 1.16 ±0.012 | | 0.81 ±0.008 | |

*"Diameter" is for diameter of lesions on soy bean leaves measured in ocular spaces. One ocular space equals 0.04 mm.

† F is for frequency of occurrence of lesions as indicated by diameter in ocular spaces to the left.

eter and direct illumination. The data obtained from these measurements are recorded in tables IX and X. They show that for all six of the experiments the average size of the lesions produced by the

*S* strain is about 1.28 times as large as that produced by the *R* strain (table XI). The *S* strain used in experiment 6 recorded in table X was reisolated from a soy bean that had been inoculated with an *R* strain. It is significant that the average size of the lesions in this experiment is 1.29 times that of the lesions caused by the *R* strain.

TABLE X

SIZE OF LESIONS PRODUCED ON SOY BEANS BY BACT. PHASEOLI SOJENSE SMOOTH AND ROUGH

| | Experiment no. 4 inoculated September 2; measured September 8, 1926 | | | | Experiment no. 5 inoculated September 6; measured September 12, 1926 | | | | Experiment no. 6 inoculated September 7; measured September 12, 1926 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Smooth | | Rough | | Smooth | | Rough | | Smooth | | Rough | |
| | Diameter* | F† | Diameter | F | Diameter | F | Diameter | F | Diameter | F | Diameter | F |
| | 12 | 2 | 10 | 5 | 12 | 1 | 9 | 1 | 12 | 4 | 9 | 3 |
| | 13 | 2 | 11 | 8 | 13 | 4 | 10 | 4 | 13 | 7 | 10 | 6 |
| | 14 | 2 | 12 | 8 | 14 | 7 | 11 | 9 | 14 | 5 | 11 | 13 |
| | 15 | 8 | 13 | 7 | 15 | 19 | 12 | 16 | 15 | 14 | 12 | 10 |
| | 16 | 8 | 14 | 12 | 16 | 10 | 13 | 16 | 16 | 14 | 13 | 20 |
| | 17 | 13 | 15 | 15 | 17 | 8 | 14 | 8 | 17 | 11 | 14 | 15 |
| | 18 | 16 | 16 | 4 | 18 | 13 | 15 | 12 | 18 | 14 | 15 | 21 |
| | 19 | 12 | 17 | 8 | 19 | 5 | 16 | 13 | 19 | 9 | 16 | 3 |
| | 20 | 7 | 18 | 9 | 20 | 12 | 17 | 6 | 20 | 13 | 17 | 15 |
| | 21 | 5 | 19 | 3 | 21 | 1 | 18 | 3 | 21 | 6 | 18 | 8 |
| | 22 | 3 | 20 | 4 | 22 | 2 | 19 | 1 | 22 | 4 | 20 | 2 |
| | 24 | 5 | 21 | 1 | 23 | 2 | 21 | 1 | 23 | 5 | 21 | 1 |
| | 25 | 2 | 22 | 2 | 24 | 3 | ..... | ..... | 24 | 2 | 22 | 1 |
| | 27 | 1 | 23 | 2 | 25 | 2 | ..... | ..... | 25 | 1 | 23 | 1 |
| | 29 | 1 | 25 | 1 | 30 | 1 | ..... | ..... | 26 | 4 | 24 | 1 |
| | 30 | 2 | ..... | ..... | ..... | ..... | ..... | ..... | 27 | 1 | ..... | ..... |
| | 32 | 1 | ..... | ..... | ..... | ..... | ..... | ..... | 30 | 2 | ..... | ..... |
| Total..... | 1692 | 90 | 1353 | 89 | 1575 | 90 | 1244 | 90 | 2106 | 116 | 1712 | 120 |
| Average size... | 18.8 ±0.14 | | 15.18 ±0.12 | | 17.5 ±0.12 | | 13.82 ±0.084 | | 18.15 ±0.12 | | 14.26 ±0.09 | |
| Average size (mm.)..... | 0.751 ±0.0056 | | 0.608 ±0.0048 | | 0.696 ±0.0048 | | 0.552 ±0.0032 | | 0.724 ±0.0048 | | 0.5700 ±0.0036 | |

* "Diameter" is for diameter of lesions on soy bean leaves measured in ocular spaces. One ocular space equals 0.04 mm.

† F is for the frequency of occurrence of lesions as indicated by diameter in ocular spaces to the left.

The probable error (23) was calculated for each set of figures of the different experiments. It is seen that the figures obtained are too small to affect the validity of the data.

The conclusions drawn from the experiments on virulence are as follows: (1) The *S* strain is more virulent than the *R* strain; it produces infection earlier and also produces larger lesions. (2) The *R*

strain can be changed to the *S* strain by passage through the soy bean plant, the *S* strain so obtained being fully as virulent as the regular *S* strain. (3) The results in these experiments demonstrate concordance between phenomena in plants with those previously reported in animal bacteriology (**1, 14, 15, 16**).

## V. Acid agglutination

The phenomenon of acid agglutination has been studied by NORTHROP and coworkers (**20, 21**), DEKRUIF (**3, 4**), and by EGGERTH and BELLOWS (**7**). The last two workers found that some

TABLE XI

SUMMARY OF DATA ON PATHOGENICITY OF BACT. PHASEOLI SOJENSE SMOOTH AND ROUGH, ON SOY BEANS AS DETERMINED BY SIZE OF LESIONS

| EXPERIMENT NO. | AGE WHEN MEASURED (DAYS) | SMOOTH | | ROUGH | | RATIO SMOOTH / ROUGH |
|---|---|---|---|---|---|---|
| | | Number measured | Average size of lesions (mm.) | Number measured | Average size of lesions (mm.) | |
| 1 | 8 | 68 | 1.61 ±0.02 | 69 | 1.28 ±0.014 | 1.25 |
| 2 | 6 | 50 | 1.26 ±0.016 | 50 | 1.03 ±0.018 | 1.22 |
| 3 | 5 | 161 | 1.16 ±0.012 | 161 | 0.81 ±0.008 | 1.43 |
| 4 | 6 | 90 | 0.751±0.006 | 89 | 0.608±0.005 | 1.23 |
| 5 | 6 | 90 | 0.696±0.005 | 90 | 0.552±0.003 | 1.26 |
| 6 | 5 | 116 | 0.724±0.005 | 120 | 0.57 ±0.004 | 1.29 |
| Total measured | | 575 | | 579 | | |
| General average | | | 1.033 | | 0.808 | 1.28 |

strains of *Bact. coli* would agglutinate in dilute acids without salts at reactions ranging from $P_H$ 1.6 to 3.0.

When the bacterial suspensions were being made for injecting the rabbits in the serological tests, it was noticed that suspensions of the rough strain of *Bact. phaseoli sojense* agglutinated in 0.85 per cent salt solution. DOCHEZ, AVERY, and LANCEFIELD (**5, 6**) found that if streptococci, which had a tendency to clump, were washed in distilled water, these would remain in suspension. In the light of their findings suspensions were again made of each of the organisms under study, and these suspensions were washed nine times in distilled water. All the organisms remained in suspension except the *R* strain, which precipitated just as rapidly after the ninth washing

as after the first. Next, suspensions of all the organisms were prepared to determine the range of $P_H$ at which they would agglutinate, and to see whether they could be differentiated on this basis.

The isoelectric point for *Bact. flaccumfaciens* no. 7 and no. 8 was found to be between $P_H$ 1.2 and 3.0 (table XII and fig. 8*a*). Obviously they cannot be differentiated on this basis. The isoelectric point of *Bact. phaseoli* no. 1 and no. 2 (table XIII and fig. 8*b*) is almost

TABLE XII

INFLUENCE OF $P_H$ ON AGGLUTINATION OF WASHED BACT. FLACCUMFACIENS IN DISTILLED WATER

| $P_H$ | BACT. FLACCUMFACIENS NO. 7 | | | BACT. FLACCUMFACIENS NO. 8 | | |
|---|---|---|---|---|---|---|
| | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 1 | Exp. 2 | Exp. 3 |
| 5.5 | − | − | − | − | − | − |
| 4.5 | − | − | − | − | − | − |
| 3.5 | − | − | − | − | − | − |
| 3.0 | − | + | − | − | + | − |
| 2.8 | + | | | + | +++ | − |
| 2.7 | | | +++ | | | ++ |
| 2.6 | + | | − | + | | − |
| 2.5 | | +++ | − | | | − |
| 2.4 | | | − | +++ | | − |
| 2.3 | | | − | | +++ | − |
| 2.2 | +++ | ++ | − | | | − |
| 2.0 | +++ | + | − | +++ | ++++c | − |
| 1.8 | ++ | + | − | ++ | ++++c | − |
| 1.6 | + | + | − | + | ++++c | − |
| 1.4 | + | − | − | + | ++++c | − |
| 1.2 | − | − | − | + | +++ | − |
| 1.2− | − | − | − | − | − | − |
| ** | $P_H$ 6.4− | $P_H$ 6.8− | $P_H$ 6.5− | $P_H$ 6.4− | $P_H$ 6.8− | $P_H$ 6.2− |

** Control suspension with no acid added.
c, Cloudy, almost complete agglutination.

identical, and does not differ materially from that of *Bact. flaccumfaciens*.

*Bact. phaseoli sojense S* was found to have a much narrower range of agglutination than strain *R;* in fact strain *R* agglutinated through practically the entire range of $P_H$ 2.0 to 9.4, little or no agglutination occurring from $P_H$ 5.5 to 5.8 (table XIV and fig. 8*c*). The spontaneous agglutination of strain *R* will easily differentiate it from all the other organisms studied.

It has been found by JACOBSON and FALK (**14**) that certain animal pathogens show a correlation between acid agglutination and the

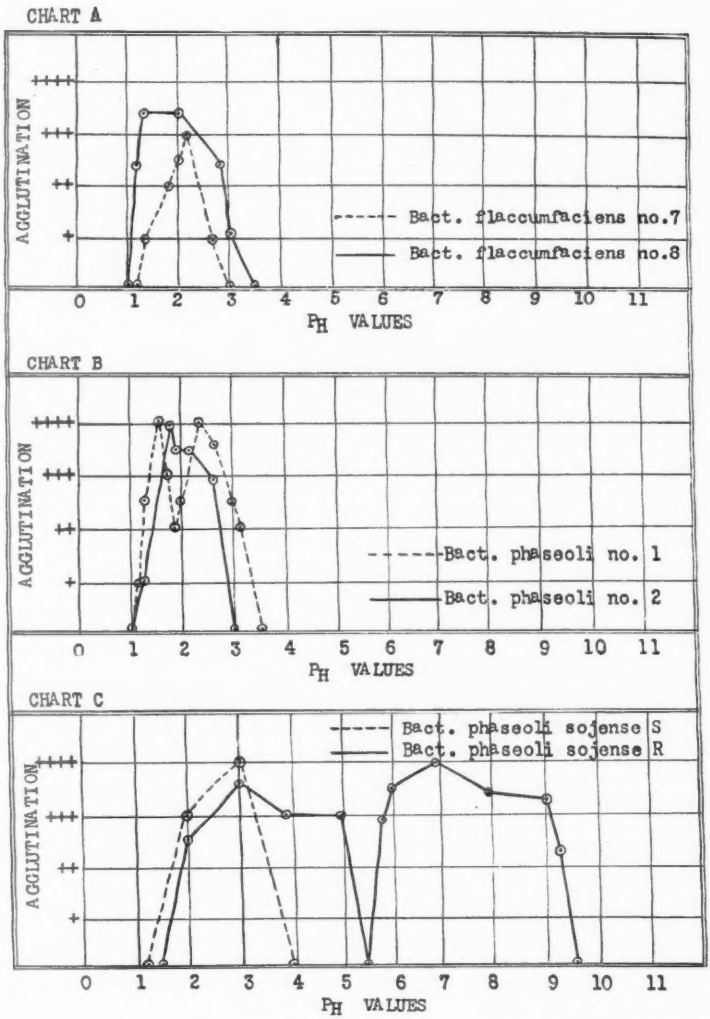


FIG. 8.—Acid agglutination of: *a*, *B. flaccumfaciens*; *b*, *B. phaseoli*, and *c*, *B. phaseoli sojense*, at various $P_H$ ranges.

## TABLE XIII

INFLUENCE OF $P_H$ ON AGGLUTINATION OF WASHED BACT. PHASEOLI NO. 1 AND NO. 2 IN DISTILLED WATER

| $P_H$ | No. 1 | | | | | | | No. 2 |
|---|---|---|---|---|---|---|---|---|
| | No. 1 | Exp. 2 | Exp. 3† | Exp. 4 | Exp. 5 | Exp. 6 | Exp. 7 | Exp. 8 |
| 6.8*.. | ..... | ..... | ..... | ..... | ..... | − | ..... | ..... |
| 6.6*.. | − | ..... | ..... | ..... | ..... | ..... | − | − |
| 6.2*.. | ..... | − | − | − | − | ..... | ..... | ..... |
| 3.1... | + | ..... | ..... | ..... | ..... | ..... | ++ | ..... |
| 3.0... | ++ | − | + | + | + | − | +++ | − |
| 2.8... | +++ | ..... | ..... | + | ..... | ..... | ..... | ..... |
| 2.6... | ..... | ++ | ++++c | ..... | ++++c | +++ | ++++c | +++ |
| 2.4... | +++ | + | ++++c | ++++c | ++ | ++++ | ++++ | ..... |
| 2.2... | + | − | ..... | ..... | ..... | ..... | ..... | ++++c |
| 2.0... | + | − | +++ | ++++c | + | ++++ | +++ | ..... |
| 1.8... | ++ | − | ++ | +++ | + | ++++ | ++ | ++++c |
| 1.7... | +++ | − | ++ | +++ | + | ++ | +++ | ++++ |
| 1.6... | ..... | − | ++ | +++ | + | − | ++++ | ..... |
| 1.4... | + | − | − | + | − | − | +++ | − |
| 1.2... | − | − | − | − | − | − | + | − |

* $P_H$ of original washed suspension before acid was added.
† Organism reisolated from the bean.
c, Cloudy, almost complete agglutination.

## TABLE XIV

INFLUENCE OF $P_H$ ON AGGLUTINATION AND ELECTROPHORETIC POTENTIALS OF WASHED BACT. PHASEOLI SOJENSE SMOOTH AND ROUGH, IN DISTILLED WATER

| WASHED | | | | | | ANOTHER WASHED SUSPENSION OF ROUGH PREPARED FROM SAME ORIGINAL, 24 HOURS LATER | | | |
|---|---|---|---|---|---|---|---|---|---|
| Smooth | | | Rough | | | | | | |
| $P_H$ | Agg. | P.D. μ/sec.† | $P_H$ | Agg. | P.D. μ/sec. | $P_H$ | Agg. | $P_H$ | Agg |
| 10..... | ..... | ..... | 10 | Dissolved | | 9.6 | + | 6.0 | + |
| 9..... | ..... | ..... | 9 | Dissolved | | 9.4 | +++ | 5.5 | − |
| 8..... | − | ..... | 8 | ++++c | −113.5 | 9.2 | ++++c | 5.0 | +++ |
| 7.0..... | − | ..... | 7.1 | ++++ | − 50.0 | 9.0 | ++++c | 4.6 | +++ |
| 6.6*..... | − | −52.2 | 6.0* | ++++c | − 97.0 | 8.9 | ++++c | 3.1 | +++ |
| 6.0..... | − | ..... | 5.8 | +++ | − 60.7 | 8.2 | ++++ | 3.0 | +++ |
| 5.0..... | − | ..... | 4.8 | +++ | − 38.6 | 7.4 | ++++c | 2.8 | +++ |
| 4.0..... | − | ..... | 3.9 | +++ | − 30.2 | 7.3 | ++++c | 2.6 | +++ |
| 3.0..... | ++++ | − 4.3 | 3.0 | ++++c | − 14.6 | 7.2 | ++++c | 2.2 | +++ |
| 2.0..... | +++ | − 5.3 | 2.0 | +++ | + 22.3 | 7.1 | ++++c | 2.0 | +++ |
| 1.2..... | − | 0 | 1.2 | ..... | ..... | 7.0* | ++++c | 1.9 | + |
| | | | | | | 6.8 | +++ | 1.8 | + |
| | | | | | | 6.6 | +++ | 1.6 | -- |

*Control; nothing added.
† P.D. determinations made by Dr. I. S. FALK.
c, Cloudy, almost complete agglutination.

electrophoretic potential difference between the organism and the suspending menstruum. In preliminary experiments a similar correlation (table XIV) between the *S* and *R* strains of *Bact. phaseoli sojense* has been found in the acid but not in the alkaline range. Additional work is in progress, and the protocol and discussion of this phase of the work will be published later.[3]

The conclusions drawn here are: (1) The isolectric point of each of these organisms is between $P_H$ 1.2 and 3.0. (2) The *R* strain of *Bact. phaseoli sojense* can be differentiated, on the basis of acid agglutination, from the *S* strain and from the other organisms as well. (3) Roughness and lesser virulence are correlated with greater agglutinability, and smoothness with greater virulence and less agglutinability. (4) For the *R* and *S* strains there appears to be a direct correlation between the P.D. and agglutination in the acid zone, with no agglutination when the P.D. is high and with complete agglutination when the P.D. is low.

## Summary and conclusions

1. A morphological, physiological, and serological study, together with virulence and acid agglutination studies have been made of *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense*.

2. Two strains, no. 7 and no. 8, were isolated from a pure culture of *Bact. flaccumfaciens*. Strain no. 7 was only slightly pathogenic for beans, while no. 8 was very pathogenic. These strains also differed morphologically in the kind of colony produced, and in appearance on agar slants and plates; otherwise they were identical both as to their physiological and serological reactions, as well as to acid agglutination.

3. Two strains, no. 1 and no. 2, were isolated from a pure culture of *Bact. phaseoli*. These differed slightly as to morphological and cultural characteristics and in virulence; but were indistinguishable serologically and by the acid agglutination test.

4. A smooth (*S*) and a rough (*R*) strain of *Bact. phaseoli sojense* were isolated from a pure culture of *Bact. phaseoli sojense* which macroscopically appeared smooth.

[3] FALK, I. S., SHARP, C. G., and LINK, G. K. K., Relation between PH, agglutination, and P.D., with *Bact. phaseoli sojense*. Proc. Soc. Exp. Biol. and Med. 1927. (In press).

5. The *S* and *R* strains were identical physiologically and serologically, but differed morphologically, in virulence, and in range of acid agglutination.

6. Colonies of the *S* strain in potato dextrose agar were always smooth, flat, and spreading, while colonies of the *R* strain were always rough and round, growing vertically instead of spreading, thereby forming umbonate colonies. On thickly sown plates umbilicate and uneven forms were noted.

7. The *S* strain remained constant throughout the investigation, neither changing in culture media nor during passage through soy bean plants.

8. The *R* strain remained constant in culture media, but when this strain was inoculated into soy bean plants and reisolations made, both *S* and *R* colonies appeared.

9. The *S* strain was more virulent for soy bean, both as to time of appearance and size of lesions. The *S* strain, reisolated from lesions produced by inoculation of the *R* strain into the plant, was fully as virulent as that recovered from lesions following inoculation with an *S* strain, and also was more virulent than the *R* strain from which it came.

10. The range of acid agglutination for the *S* strain was between $P_H$ 1.2 and 4.0, while the *R* strain agglutinated practically all the way from $P_H$ 2.0 to 9.4, except from $P_H$ 5.5 to 5.8, where little or no agglutination occurs.

11. Nine washings in distilled water by centrifuging failed to prevent the *R* strain from being spontaneously agglutinated in distilled water.

12. Greater virulence of the *S* strain seems to be correlated with motility and lesser agglutinability, and lesser virulence of the *R* strain with low motility or non-motility and with greater agglutinability.

13. In the acid zone there also appears to be a correlation between low P.D. and high agglutination, and between high P.D. and low agglutination, but not in the alkaline.

14. *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense* all differ serologically, and can be differentiated by the use of the agglutination test. *Bact. flaccumfaciens* serologically stands apart

from *Bact. phaseoli* and *Bact. phaseoli sojense*, which are quite closely related. This is in harmony with the cultural findings.

15. Culturally *Bact. phaseoli* and *Bact. phaseoli sojense* are practically identical, except for slight differences in final $P_H$ after 30 days, and in difference in character of growth in lactose and levulose broth. *Bact. flaccumfaciens* can easily be distinguished from these in all culture media used.

16. The isoelectric point for *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense* is between $P_H$ 1.2 and 3.0 for all three organisms. The isoelectric point for the *S* and *R* strains is approximately the same in the acid zone.

17. All of these organisms are more virulent to plants when the temperature is high.

The writer wishes to express his great appreciation to Dr. GEO. K. K. LINK and Dr. I. S. FALK, for their many helpful suggestions during the process of this investigation

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## EXPLANATION OF PLATE VII

FIG. 9.—Colonies of *B. phaseoli* no. 1, 8 days old, showing concentric rings and thin hyaline borders.

FIG. 10.—Colonies of *B. phaseoli* no. 1, 15 days old, showing increased number of concentric rings and thin border.

FIG. 11.—Colony of *B. phaseoli* no. 2, 8 days old, with no concentric rings or hyaline borders.

FIG. 12.—Smooth colony of *B. phaseoli sojense*, 4 days old, obtained by planting culture with needle.

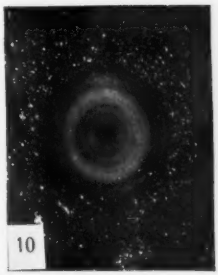
FIG. 13.—Rough colonies of *B. phaseoli sojense*, 8 days old, obtained by agar shake cultures and pouring plates.

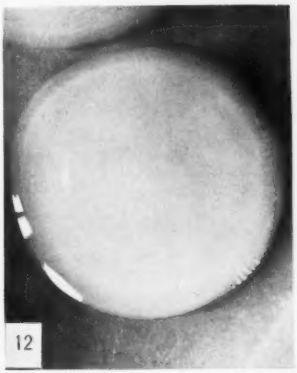
FIG. 14.—Rough colony of *B. phaseoli sojense*, 4 days old, obtained by planting culture with needle.

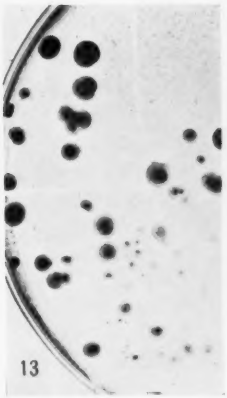
FIG. 15.—Rough colony of *B. phaseoli sojense*, 8 days old, obtained by making agar shake cultures and pouring plates; note wrinkles and umbonate form.

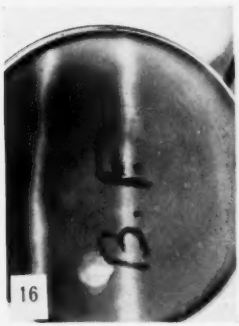
FIG. 16.—Hydrolysis of starch in 3 days by *B. flaccumfaciens*.

FIG. 17.—Hydrolysis of starch in 3 days by *B. phaseoli*.







SHARP on BACTERIUM

# CORRELATION OF HOST AND SEROLOGICAL SPECIFICITY OF BACTERIUM CAMPESTRE, BACT. FLACCUMFACIENS, BACT. PHASEOLI, AND BACT. PHASEOLI SOJENSE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 365

G. K. K. LINK AND C. G. SHARP

## Introduction

This paper and the accompanying one by SHARP have arisen out of the project of the pathology division of the Hull Botanical Laboratory for a study of the phenomena of resistance or immunity-susceptibility as seen in the field of phytopathology.[1] The phenomena of resistance or immunity-susceptibility in plants, with their vast array of many types and all degrees of biological specificity, constitute one of the most interesting and intriguing problems of theoretical and applied phytopathology. There is no need of cataloging and discussing the recorded illustrations of these phenomena. A partial enumeration has recently been made by WALKER (**39**).

Serological investigations have played an important rôle in the study of virulence and of the resistance-susceptibility phenomena in the fields of animal immunology and pathology. Animal immunologists have used plant materials to study the properties of plant protein as antigens, notably WELLS and OSBORNE (**41**), and many biochemists have studied the chemistry of these proteins following OSBORNE (**27**). In spite of the interesting and very suggestive findings by bacteriologists, zoölogists, and biochemists, plant pathologists have practically ignored serological methods in attacking their problems.

The work here reported is the result of an application of serological methods to a study of the biological specificity shown by cer-

[1] In the work reported in these papers we had the active cooperation of the department of hygiene and bacteriology of the University of Chicago, which supplied and kept the animals used. We are indebted to Dr. I. S. FALK and Dr. W. H. TALIAFERRO of that department for criticism of the data presented.

tain yellow schizomycetes which are pathogenic to plants. *Bacterium campestre*, *Bact. citri*, *Bact. malvacearum*, *Bact. phaseoli*, *Bact. juglandis*, *Bact. pruni*, and *Bact. translucens* are all yellow schizomycetes which are not readily distinguishable in culture, but which show marked biological specificity in their host relations. Thus *Bact. campestre* causes black rot of cabbage, *Bact. citri* canker of citrus, *Bact. malvacearum* angular leaf spot of cotton, *Bact. juglandis* walnut blight, *Bact. phaseoli* bean blight, *Bact. pruni* black spot and canker of peach and plum, and *Bact. translucens* black chaff of wheat.

SMITH (**34**), in discussing the pathogene of angular leaf spot of cotton, *Bact. malvacearum*, states as follows:

> In various ways this organism resembles *Bacterium campestre*, *Bacterium phaseoli*, and *Bacterium citri* but I did not succeed in cross inoculating it to cabbages, to beans, or to oranges. Further comparison should be made not only with *Bacterium campestre*, *Bacterium citri*, and *Bacterium phaseoli*, but also with *Bacterium pruni*, *Bacterium juglandis*, and *Bacterium translucens*, all of which are closely related. Indeed, some of the names are perhaps synonymous, but this can be settled only by many cross inoculations and much further study.

In his discussion of *Bact. phaseoli*, the pathogene of blight of beans, he writes:

> On a variety of culture media *Bacterium phaseoli* is closely like *Bacterium campestre*, but the two organisms are not identical, as shown by the failure of repeated cross inoculations (cabbage bacterium on beans and bean bacterium on cabbages), but our present means of separating the two forms culturally is insufficient. It is also culturally much like *Bacterium citri*, but with a virulent strain I failed to obtain any scabs in *Citrus decumana* (about 60 young seedlings). The student, therefore, who has opportunity might direct his attention to comparative studies of the yellow organisms of this group in the hope of finding additional differences by the use of new media. But in any event, *Bacterium phaseoli* belongs with *Bacterium campestre*, *Bacterium hyacynthi*, *Bacterium vascularum*, *Bacterium pruni*, *Bacterium malvacearum* (no. X), *Bacterium citri*, and *Bacterium translucens* in a closely related kinship.

It is obvious from these quotations that there is apparent biological specificity in the pathogenes discussed, so far as host relations are concerned, and that in the main this has been the reason for considering these organisms as distinct species. The same problem in a more refined state exists, so far as various bean pathogenes are concerned, where we have *Bact. phaseoli*, the pathogene of bean blight, *Bact. flaccumfaciens*, the cause of bean wilt, and *Bact. phaseoli so-*

*jense*, the pathogene of bacterial pustule of the soy bean. This situation has been investigated by SHARP in the accompanying paper.

It seemed worth while to determine whether serological technique, specifically the agglutination test, could be used to differentiate these microorganisms, and possibly in addition to tell something about their relationship. A positive result would give another and needed proof that these organisms are deserving of species rank. More important theoretically, however, was the consideration that if we should find serological specificity correlated positively with biological specificity, so far as host relations are concerned, there would be some promise that serological technique might become a tool for the study of resistance-susceptibility and virulence phenomena in phytopathology. We were thus carried into the field of sero-diagnosis, which has been used extensively by bacteriologists and animal immunologists, but which botanists with a few exceptions have left practically untouched. A perusal of the literature on sero-diagnostic methods applied to microorganisms, in the main, promised that we might be successful; but there also were indications that we might fail.

GRUBER and DURHAM (**12**), while studying the bactericidal reaction with colon bacilli and cholera spirilla, noticed that homogeneous suspensions of these microorganisms were agglutinated or clumped by their own or homologous antisera, but not by heterologous sera. They recognized the specificity of the reaction, and proposed that the method could be used for bacterial differentiation and species determination. This method (the agglutination or agglutinin test) has become a very important tool of almost universal application in the hands of the immunologist and bacteriologist, and is used most frequently for rapid identification of colonies of doubtful typhoid or dysentery. By this method the dysentery bacillus was discovered by SHIGA. The Gruber-Widal test, which is the converse of the preceding method, is used generally for the early diagnosis of typhoid fever. The agglutination test has also been used to differentiate and classify microorganisms which are impossible to separate by means of cultural, inoculation, and microscopic tests. Thus, BUTTERFIELD and NEILL (**5**) have demonstrated that all agglutinable strains of meningococci may be classified under four types.

JONES (15) showed that sixteen strains of *Bacillus bovisepticus* can on serological and cultural grounds be put into three groups. On the other hand, Miss EVANS (7) found that serologically she could not differentiate the strain of *Brucella melitensis*, which is associated with malta fever in man, and the strain associated with abortion in cattle.

The discovery of GRUBER and DURHAM led to experimentation which demonstrated that agglutination or clumping could be brought about, not only by antisera produced by injection into suitable animals of whole bacteria as GRUBER and DURHAM had done, but also by injection of dissolved extracts of bacteria or of filtrates of the old culture media. Following this line of investigation, KRAUS (18) found that clear filtrates of the broth cultures of *Bacillus pestis* and the cholera spirillum, when mixed with their respective antisera, gave at first turbidity and finally a precipitate. He found that the reaction was specific, and pointed out the practical diagnostic possibilities of the discovery. He named this the "precipitin reaction," having thus discovered the precipitation or precipitin test which also has become extremely important in serological technique.

This method has been used in the diagnosis of glanders by WLADIMIROFF, and is an important adjunct to other methods in determining the biological relationship of microorganisms. Its greatest practical use, however, is outside the field of microorganisms, because it was soon found that specific antisera are produced whenever animals are injected with any kind of foreign proteins, bacterial, plant, or animal. Practically, the precipitin test is used extensively in forensic medicine and in determining the adulteration of meat products and of meals (flour). In theoretical biology it has been used to determine phylogenetic relationship of animals, especially by NUTTALL (26), and of plants most extensively by MEZ and his coworkers (24), and by GOHLKE (11).

The relative infrequency of bacterial diseases of plants in Europe, as well as long delayed recognition by German bacteriologists of the rôle of schizomycetes as plant pathogenes, probably were factors responsible for the fact that serological technique was not at once applied to these organisms by bacteriologists and botanists when the wave of serological testing swept through the ranks of biologists and the medical profession in Europe. As a result, the first attempts to

use the new technique in the botanical field outside of bacteriology were made in connection with experiments to determine whether the precipitation test could be employed to detect adulteration in flours and meals. Following the earlier work of KOWARSKI (**17**) and BERTARELLI (**3**), which opened the way but which was not conclusive, a considerable number of investigators have touched this field, especially MAGNUS (**22,23**), MAGNUS and FRIEDENTHAL (**21**), and RELANDER (**29**). WENDELSTADT and FELLMER (**40**) used the precipitin as well as the complement fixation test. SAULI (**31**) employed the conglutination test. GASIS (**9**) and BECKER (**2**) introduced more refined quantitative methods, and as a result the latter found that he could detect an adulteration of 0.125 per cent (0.000001 gm.) of *Argostemma Gigathi* in wheat flour. He also applied the method to detection of foreign seeds in seed stocks. More recently these methods have been used in connection with genetic studies in separating and identifying strains and varieties of agricultural plants, especially by ZADE (**43**) and ARZT (**1**).

SCHÜTZE (**32**) in 1903 attempted an application of serological technique to a mycological problem. He endeavored to differentiate races or varieties of yeasts, but without success. CITRON (**6**) tried to apply the methods to differentiate the *Favus* from the *Trichophyton* fungi, but without success. The first encouraging results in the application of serological technique to problems of this type were obtained by MAGNUS and FRIEDENTHAL (**20**). These investigators endeavored to determine whether serological test methods would support the deductions of morphological systematists as to relationship of fungi based on comparative morphology. They used extracts of *Saccharomyces cerevisiae*, *Tuber brumale*, and *Agaricus campestris* as antigens. Their findings corroborated the current opinion of the relationship of these three fungi.

ROSENBLATT-LICHTENSTEIN (**30**) in 1912 made serological tests using, in pure culture, six strains of algae, whose identity, however, was not determined. In 1916 LIESKE (**19**) applied serological methods to a study of unicellular green algae. He made use of all the methods, agglutination, precipitation, and complement fixation, obtaining especially good results with agglutination and complement fixation. He found, however, that the tests were species specific only

in certain dilutions. When proper precautions were taken as to dilutions, the agglutination test enabled him to differentiate races or strains of algae which had arisen in his pure cultures as a result of varying the nutrient medium and illumination. His final conclusion was that rerological methods can be made an exceedingly valuable adjunct in modern algology. Much later GUTTMAN (**13**), in applying serological technique in phylogenetic studies of the archegoniates, attempted to use *Spirogyra* against the immune sera of several archegoniates. He was unsuccessful because the protein extract (1:200 in 0.85 per cent physiological salt solution) of this alga became turbid after incubation for some time. STEINECKE (**35**) repeated part of LIESKE'S work, using the agglutination, precipitation, and conglutination tests. In harmony with LIESKE, he concluded that the agglutination test is suitable for studies of unicellular algae, but he carried the work much further. He set himself the task of determining whether serological methods could be used to determine phylogenetic relationships in the algae, and, if this proved feasible, to use the results of the test to construct a phylogenetic tree of these forms. He did not use complement fixation because it is especially suitable for diagnosis of species, and he was interested primarily in determining large relationships. Consequently he used the precipitin and conglutination tests. After working out details as to methods of preparing protein extracts, dilutions, etc., he obtained very striking results.

In the meantime serological methods were finding their way to pathological and phytophysiological investigations. The first as well as the most extensive work, although dealing with a phytopathological problem, was not done by phytopathologists, but by soil bacteriologists interested in the bacteria of the nodules of legumes. ZIPFEL (**44**) seems to be the first one to have made such studies. He showed a serological relation between the strains of the organisms found in the nodules of *Pisum sativum* and *Phaseolus vulgaris* on the one hand, and between *Trifolium pratense* and *Vicia faba* on the other hand, but he found a distinction between these two strains. KLIMMER and KRÜGER (**16**) employed the agglutinin, complement fixation, and precipitin tests to eighteen distinct strains of legume nodule bacteria. They found the serological characteristics of the

strains to be definite, and were able to correlate them with cross-inoculation of host plants. VOGEL and ZIPFEL (**38**) showed that legume nodule organisms probably are not one species, that different types can be differentiated by both agglutination and precipitation, and that these methods can be used for differentiation of legume nodule bacteria from other soil bacteria.

STEVENS (**36**) made a careful study of fifty-five strains of nodule bacteria representing the seven groups of cultivated legumes. His results confirm those of KLIMMER and KRÜGER and others as to host plant groups, and serological properties of the bacteria. In addition he showed that the strains of the same host could be separated into distinct serological groups. SIMON (**33**), in studying the nodule bacteria of seven genera of Leguminosae, found five cross-inoculating and cross-agglutinating groups. BIALOSUKNIS and KLOTT (**4**) found that the serological properties of strains remained constant, that more than one serological strain may form nodules on one plant, but that only one serological type is found in each nodule. WRIGHT (**42**), in studying the *A* and *B* types of *Pseudomonas radicicola* of the soy bean (*Soja max*), found that the strains of the organism are distinct serologically, that the serological characters are constant, and that they are not changed by continued artificial culture or by plant passage. He found, however, that the serological properties of these organisms cannot be used to identify any strain of the organism or the types. FRED, WHITING, and HASTINGS (**8**), using the agglutination test, found that serologically they could subdivide cultures of legume nodule bacteria belonging to one cross-inoculation group. Upon these differences in agglutination they based their selection of strains used in a study of nitrogen assimilation.

JENSEN (**14**), an animal pathologist, applied the agglutination test to a study of strains of *Bacterium tumefaciens* isolated from the Paris daisy. He was able to differentiate between the strain he had isolated from the daisy in Denmark and the strain SMITH had isolated from the daisy in America. An excellent piece of work in the application of serological technique to phytopathological problems has been done by NELSON (**25**), a bacteriologist. He found that he was able, by serological methods, to distinguish between the proteins of a variety of flax resistant to wilt (*Fusarium lini*) and a variety

not resistant to the wilt. PAINE and LACEY (**28**) applied the agglutination test to *Bacillus lathyri*, *Bacterium phaseoli*, and *Aplanobacter michiganense*. Although the first two gave group agglutination, they concluded that these organisms could be differentiated by this method. However, strains were found intermediate between *B. lathyri* and *Bact. phaseoli* that agglutinated equally well with the two sera. They suggest that possibly one species had arisen from the other in the tissue of the plant. *Aplanobacter michiganense* failed to agglutinate by either serum or by its own homologous serum. TAKIMATO (**37**) reported that serological tests indicate that the bacteria isolated from soft rot of celery, lettuce, and radish belong to the same strain, although the radish organism differs from the others when grown in culture media.

GOLDSWORTHY (**10**) reports the formation of specific agglutinins, with a titre of 1/10,000, in rabbits following intravenous injection of *Bact. maculicolum* (cauliflower pathogene). He used these agglutinins to determine the presence of *Bact. maculicolum* in the soil.

MEZ (**24**), in presenting the results of fifteen years' work in the use of sero-diagnosis for determination of phylogenetic relationships of plants, expresses doubt whether serological methods can meet the extreme demands made upon them, by bacteriologists in particular, for the differentiation of strains of microorganisms or of closely related species. He states:

> Hier sei nur betont, das eine gewisse Ernüchterung der Bakteriologen bezüglich der Serodiagnostik, die sich mehrfach bis zum Zweifel an der Spezifität der Methode gesteigert hat, darauf zuruckzuführen ist, dass zuviel von ihr verlangt worde. Die Unterscheidung z.B. von, "Paratyphus 1,2 und 3" oder ähnlich nächstverwandter Formen geht mit Hilfe der Eiweissdifferenziation nicht, jedenfalls nicht mit Sicherheit. Ebenso bin ich bezüglich der Ergebnisse von Arzt (Mez, Archiv XIII [1926], S. 117ff.), welcher mit Hilfe der genauesten Dosierung der reagierenden Eiweissmengen bei der Untersuchung der Phylogenie der Gerstenvarietaten Erfolg haben will, noch etwas skeptisch.

## Experimentation

The work reported in this paper is restricted to a study of *Bact. campestre*, the crucifer pathogen, and to the bean pathogenes *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* which SHARP used in his studies. The value of our results would have been en-

hanced had we been able to include other yellow schizomycetes which are similar to *Bact. campestre* and *Bact. phaseoli*.[2]

We decided to use first the simplest serological methods (agglutination and precipitation tests), and, if these failed, to resort to the much more complicated complement fixation test. Since we obtained striking results with the agglutination test, we merely applied the precipitation test to determine whether broth cultures of these organisms could be used in making a precipitin test (see accompanying paper by SHARP). The complement fixation test was not used at all.

The cultures of the organisms used were tested for their pathogenicity both before and at the close of the experiment recorded here. Each organism was grown on standard potato dextrose agar slants ($P_H$7.2) for four days. Even suspensions were then made of each organism in 50 cc. of 0.85 per cent NaCl solution. These suspensions were used as stock antigens for injection of rabbits and production of antisera. Rabbits were used for production of antisera, two animals being used for each organism and as sources of normal serum. Four intravenous injections of the organisms were made into the ear vein of each of the rabbits at intervals of four days, using 0.5 cc. for the first, and 1 cc. for each of the remaining three injections. Ten days after the last injection the rabbits were bled from the heart, the sera prepared, and kept under aseptic conditions until they were used in the agglutination tests.

The cultures used for preparation of the suspensions in the agglutination tests were grown on standard beef extract-peptone-dextrose agar ($P_H$ 7.2). The organisms were washed off the agar with a 0.85 per cent salt solution, and then shaken thoroughly in the saline solution and centrifuged. The supernatant liquid was poured off and the organisms were washed and centrifuged a second time, the last suspension in saline solution being used for the agglutination tests. Suspensions of *Bact. campestre*, *Bact. phaseoli*, *Bact. phaseoli sojense* (rough), and *Bact. flaccumfaciens* are centrifuged out of suspension much more readily than *Bact. phaseoli sojense* (smooth). The organisms were grown on different media and washed in saline to take pre-

[2] This has now been done by LINK and LINK. Paper in manuscript and will appear in BOT. GAZ. 1927.

caution against a positive precipitin reaction with the protein of the potato agar used in preparing the antigen.

The sample protocol of table I shows the set-up used in each experiment: 0.4 cc. of antiserum was added to 0.6 cc. of saline solution (0.85 per cent NaCl), giving a dilution of 1-2.5. From this original dilution the other dilutions were obtained by mixing and then transferring 0.5 cc. of the diluted antiserum from tube 1 to tube 2 which contained 0.5 cc. of saline, from tube 2 to tube 3, and so on, up through tube 10 or 11. After thoroughly mixing the contents of

TABLE I

TYPICAL PROTOCOL OF AGGLUTINATION TESTS

| | Tubes | | | | | | | | | | | Control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | |
| Antiserum: Dilution | 1–2.5 | 1–5 | 1–10 | 1–20 | 1–40 | 1–80 | 1–160 | 1–320 | 1–640 | 1–1280 | 1–2560 | ..... |
| Antiserum: Amount in cc | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0 |
| Bacterial suspension (antigen) in cc | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Saline solution (0.85 per cent NaCl) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 |
| Final dilution of antiserum | 1–5 | 1–10 | 1–20 | 1–40 | 1–80 | 1–160 | 1–320 | 1–640 | 1–1280 | 1–2560 | 1–5120 | ..... |

each tube, 0.5 cc. of bacterial suspension in saline solution (antigen) was added to each tube, thus giving a dilution of 1–5 in tube 1, up through dilution 1–5120 in tube 11. The control in each set-up consisted of a tube containing 0.5 cc. saline and 0.5 cc. of bacterial suspension in saline. After thorough mixing by shaking, the series of tubes was incubated for 1 hour at 37° C. in a Wasserman bath and then placed in a refrigerator for 12 hours. Complete clearing of the suspension by agglutination is indicated by ++++, partial agglutination by +++ and ++, and the last definitely detectable agglutination by +; ± indicates that there was suggestion of a clumping.

As a control for all series, the experiment was also done using the serum of a normal animal against the suspensions of the organisms tested. Unfortunately one animal was lost, so that there was the normal serum of only one rabbit for a control. The results of the experiments are given in tables II, III, and IV.

Slight agglutination occurred in very low dilutions when normal serum of one rabbit was used against suspensions of the organisms

TABLE II

AGGLUTINATION TESTS USING NORMAL SERUM AGAINST SUSPENSIONS OF BACT. CAMPESTRE, BACT. PHASEOLI, BACT. PHASEOLI SOJENSE, AND BACT. FLACCUMFACIENS

| DILUTION OF SERUM | BACT. CAMPESTRE | BACT. PHASEOLI | BACT. PHASEOLI SOJENSE | BACT. FLACCUM-FACIENS |
|---|---|---|---|---|
| 1–5 | ++ | + | + | + |
| 1–10 | + | + | + | + |
| 1–20 | + | ± | ± | ± |
| 1–40 | − | − | − | − |
| 1–80 | − | − | − | − |
| 1–160 | − | − | − | − |
| 1–320 | − | − | − | − |
| 1–640 | − | − | − | − |
| 1–1280 | − | − | − | − |
| 1–2560 | − | − | − | − |
| 1–5120 | − | − | − | − |
| 1–10240 | − | − | − | − |
| Saline | − | − | − | − |

TABLE III

AGGLUTINATION TESTS USING ANTISERUM OF BACT. CAMPESTRE AGAINST BACT. CAMPESTRE, BACT. PHASEOLI, BACT. PHASEOLI SOJENSE, AND BACT. FLACCUMFACIENS

| DILUTION OF SERUM | BACT. CAMPESTRE | BACT. PHASEOLI | BACT. PHASEOLI SOJENSE | BACT. FLACCUM-FACIENS |
|---|---|---|---|---|
| 1–5 | ++++ | + | + | + |
| 1–10 | ++++ | + | + | + |
| 1–20 | ++++ | + | + | + |
| 1–40 | ++++ | − | − | ± |
| 1–80 | ++++ | − | − | − |
| 1–160 | ++++ | − | − | − |
| 1–320 | ++++ | − | − | − |
| 1–640 | ++++ | − | − | − |
| 1–1280 | ++++ | − | − | − |
| 1–2560 | ++++ | − | − | − |
| 1–5120 | +++ | − | − | − |
| 1–7680 | + | − | − | − |
| 1–10240 | − | − | − | − |
| Saline | − | − | − | − |

(table II). This probably was due to an idiosyncrasy of the normal serum of this animal, because a test made against *Bact. phaseoli*

with the remainder of the normal serum used by SHARP gave no agglutination at all. Consequently it probably is permissible to ignore the slight agglutination noted in dilutions 1-5 to 1-20, especially since doing so does not essentially alter the data or the conclusions. It would have been better and the results absolutely conclusive if we had, as we are now doing, bled each rabbit before beginning the injections to determine the reaction of the normal serum with the antigen used.

TABLE IV

AGGLUTINATION TESTS USING ANTISERA OF BACT. PHASEOLI, BACT. PHASEOLI SOJENSE, AND BACT. FLACCUMFACIENS AGAINST HOMOLOGOUS ORGANISMS AND AGAINST BACT. CAMPESTRE

| DILUTION OF SERUM | ANTISERUM OF BACT. PHASEOLI VS: | | ANTISERUM OF BACT. PHASEOLI SOJENSE VS: | | ANTISERUM OF BACT. FLACCUMFACIENS VS: | | NORMAL SERUM VS: |
|---|---|---|---|---|---|---|---|
| | BACT. CAMPESTRE | BACT. PHASEOLI | BACT. CAMPESTRE | BACT. PHASEOLI SOJENSE | BACT. CAMPESTRE | BACT. FLACCUM-FACIENS | BACT. CAMPESTRE |
| 1–5 | ++++ | ++++ | +++ | ++++ | +++ | ++ | ++ |
| 1–10 | ++++ | ++++ | ++ | ++++ | ++ | +++ | + |
| 1–20 | +++ | ++++ | ++ | ++++ | + | ++ | + |
| 1–40 | ++ | ++++ | ++ | ++++ | − | ++ | − |
| 1–80 | + | +++ | + | ++ | − | ++ | − |
| 1–160 | ± | ++ | ± | + | − | + | − |
| 1–320 | − | + | ± | + | − | − | − |
| 1–640 | − | + | − | ± | − | − | − |
| 1–1280 | − | ± | − | − | − | − | − |
| 1–2560 | − | − | − | − | − | − | − |
| Saline | − | − | − | − | − | − | − |

The antiserum of *Bact. campestre* (table III) was highly specific for its homologous organism, giving complete agglutination through the dilution range of 1-5 to 1-2560, but gave only a slight (++ or +) agglutination in low dilutions with suspensions of the heterologous organisms against which it was tested. The titre obtained against the homologous organism was 1-7680.

Table IV shows that when the antisera of *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* were used against suspensions of their homologous organisms, specific agglutination occurred. *Bact. phaseoli* gave a titre of 1–1280, with complete agglutination in dilution 1–5 to 1–40. *Bact. phaseoli sojense* gave a titre of 1–640, with complete agglutination in dilution 1-5 to 1-40. The antiserum of

*Bact. flaccumfaciens* gave a titre of 1-160 but no complete agglutination at any dilution. *Bact. phaseoli* and *Bact. phaseoli sojense* gave agglutination with *Bact. campestre* in dilutions 1-160 and 1-320 respectively, while antiserum of *Bact. flaccumfaciens* gave agglutination with *Bact. campestre* in dilution 1-20. Consequently in low dilutions, group agglutination was obtained when antisera of *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* were used against *Bact. campestre*.

On the basis of current interpretations of many immunologists, these results indicate that although *Bact. campestre* contains some antigens (proteins) which stimulate the production of the same antibodies as the bean pathogenes studied, that is, *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens*, in addition it contains others which stimulate the production of different antibodies from the bean pathogenes. Clearly, therefore, *Bact. campestre* can be differentiated specifically from the bean pathogenes on this basis. The group agglutination, shown when antisera of the bean pathogenes are used against suspensions of *Bact. campestre* in low dilutions (table III), indicate that *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* contain antigens (proteins) which cause the production of antibodies that react with *Bact. campestre*. The slighter agglutination obtained when antiserum of *Bact. flaccumfaciens* was used against *Bact. campestre* indicates that the former organism does not contain as many antigens in common with *Bact. campestre* as *Bact. phaseoli* and *Bact. phaseoli sojense*. On the basis of these interpretations, *Bact. campestre* is closely related to *Bact. phaseoli* and *Bact. phaseoli sojense*, although it is distinct from them, while it is less related to *Bact. flaccumfaciens*.

## Conclusions

1. The agglutination test can be used to differentiate *Bact. campestre* from *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens*.

2. Serologically *Bact. campestre*, although distinct, is closely related to *Bact. phaseoli* and *Bact. phaseoli sojense*. It is less related to *Bact. flaccumfaciens*.

3. The biological specificity shown by *Bact. campestre*, *Bact. phaseoli*, *Bact. phaseoli sojense*, and *Bact. flaccumfaciens* in their host relations is correlated with immunological or serological specificity.

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# RELATION OF DESICCATING WINDS TO FLUCTUATIONS IN ASH CONTENT OF CITRUS LEAVES AND PHENOMENON OF MOTTLE-LEAF[1]

A. R. C. HAAS AND H. S. REED

(WITH THREE FIGURES)

## Introduction

The vegetation of southern California is exposed to severe desiccating winds during the months of October, November, and December. Their meteorology has been discussed by BLAIR (1), MCADIE (4), and CARPENTER (3). Often the winds blow continuously for several days, but at times intermittently, thus giving the trees an opportunity to recover between the windstorms. The hot winds may strip leaves from the trees, wilt, or scorch them. Leaves will recover from the wilted condition unless the wind persists for some time, but scorched leaves are killed without much preliminary wilting (fig. 1).

It has been found that the mature orange leaf has less resistance to excessive water loss than the leaves of many other trees (2). Apparently the stomata of the older leaves have little power to protect against such severe losses. Whether the scorching of leaves is due to an insufficient supply of water in the soil, or to inability of the conducting systems to carry sufficient water to the leaves has not been determined; probably both factors play a part.

Scorched orange leaves may remain attached to the tree for some time and continue to lose water (fig. 1). Leaves which have been severely wilted often have areas killed although most of the leaf recovers (fig. 2). If the dry hot winds are sudden and severe, leaves that are struck directly, in an unprotected grove or in the tops of tall trees, may be killed almost immediately without the loss of very much water. So long as the drying leaf remains attached there is likelihood of a slight loss of moisture through the old leaf. Frequently the wind blows severely for a few hours, causing leaves to become badly wilted, but the subsequent calm permits partial recovery. The

[1] Paper no. 153, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

winds may again resume their activity, and during several days there may be successions of wind and calm. Ordinarily such brief winds do not cause severe injury except to strip the leaves from the tree mechanically.

If the leaves have been killed, new ones arise in the leaf axils. Some important internal action is going on under the influence of the wind to annul the external action, according to the theory of LE CHATELIER. As the wind blows and evaporates moisture from the leaves, the concentration of salts increases, which serves to raise the osmotic pressure of the cell sap and presumably to reduce the rate of transpiration. It is conceivable that frequent but brief winds of moderate intensity may raise the salt content of the leaf cells to a point where they are better able to withstand prolonged desiccation by subsequent winds. It has been noted that the worst effects are usually produced by severe winds in early autumn.

FIG. 1.—Orange twig bearing scorched leaves; photograph shows adherence of leaves a month after they were killed.

When the wind ceases and the transpiration rate falls, the wilted leaves regain their turgor and normal salt content. The excessive salts are for the most part redistributed in other portions of the tree or returned to the soil solution. If leaves are killed soon after the wind strikes them, they lose but little water prior to this and but little salt accumulates within them. If the leaves are killed after the salt accumulation has reached a high level, however, their loss is probably more injurious to the shoots and other portions of the tree, because they are thus deprived of some of their salts.

The loss of calcium through wind defoliation may be inferred from the mottled condition of the next set of leaves (fig. 3); and the

loss of organic nutrients such as carbohydrates and proteins may be inferred from the small size of the subsequently formed leaves.

## Experimentation

The effect of desiccating winds upon the composition of mature orange leaves has been studied, using material from trees growing in soil in lysimeters 4 feet deep and 8 feet in diameter, with a drainage

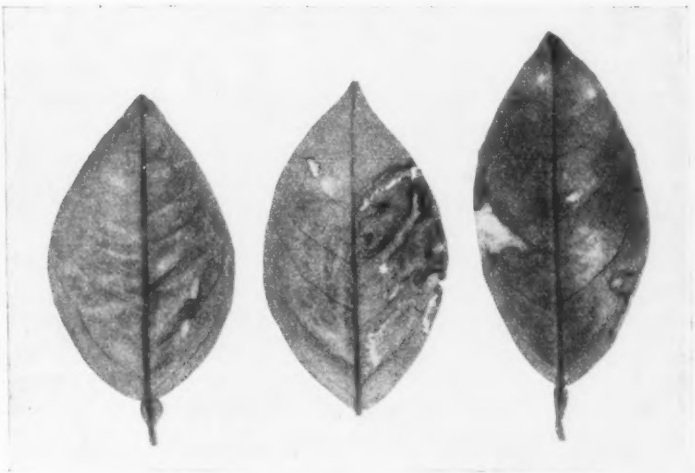

FIG. 2.—Dead areas on orange leaves due to temporary drought conditions; specimens came from trees in pots of soil, but similar injury may be seen in groves not sufficiently irrigated.

pipe from the lowest point of the tank. The soil was carefully irrigated to maintain a desirable moisture content without the use of an excess of water.

The weather records were kindly furnished by the Department of Orchard Management of this Station. Following a period of cool humid weather, a dry wind began to blow on December 9, 1924. The temperature began to rise and the humidity to fall as the wind velocity increased. Some of the data on atmospheric conditions are given in table I. The wind velocity on December 11 at times was more than 25 miles per hour, but the temperature was higher on the

next day and the cumulative effect of the desiccating factors was then reached.

Within an hour certain leaves, struck directly by the wind, were dry as powder, although still attached to the tree. Such leaves were killed so quickly that no appreciable amount of salt accumulated in them. Others were badly wilted even though the soil was sufficiently moist. Mature leaves that were killed and also those that were wilted were collected on December 12, 1924, within a few hours after the wind began. When the wind ceased the wilted leaves regained

TABLE I

CLIMATOLOGICAL DATA FOR DAYS ON WHICH LEAF SAMPLES WERE COLLECTED

| ATMOSPHERIC CONDITION | DECEMBER 1924 | | | | |
|---|---|---|---|---|---|
| | 10 | 11 | 12 | 21 | 22 |
| Temperature maximum (° F.) | 69 | 73 | 80 | 60 | 64 |
| Temperature minimum (° F.) | 41 | 41 | 46 | 32 | 35 |
| Relative humidity (per cent) at 8:00 A.M. | 22 | 20 | 19 | ..... | 79 |
| Lowest humidity for 24 hours | 15 | 12 | 16 | 27 | 46 |
| Average wind velocity (miles per hour) | 10.1 | 17.8 | 11.5 | ..... | ..... |
| Maximum wind velocity | 22 | 26 | 20 | 2 | 4 |
| Hours of sunshine | 7.17 | 7.34 | 8.83 | 5.92 | ..... |

their turgidity, and on December 22 another sample of mature leaves was taken. Table II compares the wilted leaves of December 12 and the turgid ones of December 22 (uninjured), with the quickly desiccated leaves of December 12 (injured). Analysis was made of the water solubility of the ash constituents of the dry matter, and in some cases total nitrogen determinations were made. The injured leaves, when pulverized and floated on distilled water, appeared to be buoyant for a much longer time than uninjured leaf material. The dry wind probably coagulated the protoplasm of the cells and thus made possible an increased content of air in them, which lowered the specific gravity of the material and therefore caused it to remain at the surface.

The leaf samples were dried at 60°–70° C. Table II gives the results of analyses of the samples obtained from trees grown in tanks of soil that were fertilized with calcium nitrate. On December 12 the

uninjured leaves had accumulated considerable salts and the injured ones very little. The point should be emphasized that this accumulation was not permanent but was dependent upon the occurrence of the wind. The winds did not blow to any extent from December 12 to 22, and the figures for December 22 show that the ash had returned to approximately what it was before the wind began. The point of interest is that there may be a temporary accumulation of ash constituents in the leaves instead of a permanent one, and that

TABLE II

EFFECT OF DESICCATING WINDS UPON COMPOSITION OF VALENCIA ORANGE LEAVES FROM TREES WHERE CALCIUM NITRATE HAD BEEN APPLIED TO SOIL; RESULTS EXPRESSED AS PERCENTAGE OF DRY MATTER

| | December 12, 1924 | | December 22, 1924 Uninjured | December 12, 1924 Injured | | December 12, 1924 Uninjured | |
|---|---|---|---|---|---|---|---|
| | Injured | Uninjured | | Water insoluble | Water soluble | Water insoluble | Water soluble |
| Ash | 14.10 | 17.03 | 14.58 | 6.10 | 8.11 | 6.26 | 10.31 |
| Ash constituents: | | | | | | | |
| Na | 0.14 | 0.14 | 0.18 | ..... | 0.16 | 0.03 | 0.18 |
| K | 0.60 | 0.50 | 0.60 | 0.04 | 0.68 | 0.02 | 0.53 |
| Ca | 4.51 | 5.76 | 4.83 | 2.08 | 2.39 | 2.26 | 3.26 |
| Mg | 0.40 | 0.42 | 0.38 | 0.08 | 0.31 | 0.08 | 0.40 |
| Cl | 0.03 | 0.04 | ..... | Trace | 0.03 | Trace | 0.04 |
| $SO_4$ | 0.40 | 0.33 | 0.30 | 0.09 | 0.26 | 0.05 | 0.26 |
| $PO_4$ | 0.61 | 0.56 | 0.54 | 0.15 | 0.32 | 0.16 | 0.43 |

the ash content of citrus leaves in the field may depend not only upon their age, but also upon the severity of external climatic conditions. If the winds blew continuously the ash content of the leaves might remain relatively high. It seems logical to conclude that the salt requirement of the leaves must have been satisfied prior to the increase due to the wind; otherwise there would be no such return to the original concentration as is found after a period of calm weather (table II).

The effect of the wind seems to be especially reflected in the calcium content (table II). In uninjured leaves the calcium is always considerably increased by the wind, and if such leaves are killed before the wind ceases blowing it can readily be understood why the shoots should be depleted of soluble calcium for the subse-

quent new growth. This is probably a potent cause of much of the mottle-leaf which occurs on trees exposed to desiccating winds. In spite of the fact that the soil was rich in soluble calcium, the shoots

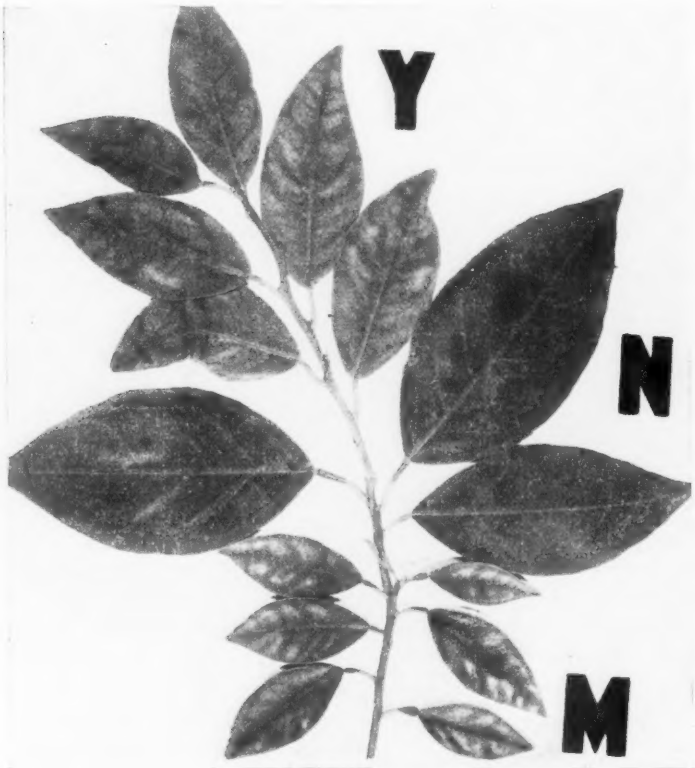


FIG. 3.—Leaves on orange twig defoliated by desiccating winds: *M*, small mottled mature leaves on shoot whose first crop of leaves was killed by wind; *N*, normal leaves on succeeding cycle of growth; *Y*, young leaves on last cycle of growth, still immature but healthy

in many cases were depleted of it through the loss of leaves. Since percolates from these lysimeters contained 1300–2500 p.p.m. Ca on September 24, 1924, it seems safe to assume that there was no calcium deficiency in these soils. Fig. 3 shows strikingly the mottled,

dwarfed leaves on shoots which had suffered this type of defoliation. After the trees were adequately protected by windbreaks, the shoots produced healthy large leaves on the next cycle of growth. It seems, therefore, that the trees were unable to acquire sufficient calcium from the soil to produce healthy leaves, at least during the period immediately following defoliation. At that season the new growth of the orange tree seems largely dependent upon the supplies of soluble materials already in the shoots.

The data in table II also show that the increase in salt content of the leaves was due to an increase in the water soluble portion. The increase of water soluble ash from 8.11 to 10.31 per cent is very significant. The uninjured survivors showed an increase in water soluble calcium from 2.39 to 3.26 per cent of the dry matter, but the increase in insoluble calcium was relatively small. The writers have shown (**6**) that the amount of calcium expressed as a percentage of the ash for leaves, shoots, and trunks was 28.16, 28.01, and 23.51 respectively. Calculated as a percentage of dry matter, this would be 3.93 for leaves, 1.65 for shoots, and 0.55 for trunks. The fractions which were soluble in water varied greatly, being 45.82 per cent for leaves, 15.03 per cent for shoots, and 8.69 per cent for trunks. These data show that the shoot and trunk contain relatively less calcium than the leaves, and, what is still more important, that it is relatively much less soluble in water. We have also shown (**5**) that orange trees grown without sufficient calcium seemed to be unable to utilize calcium which was present in the trunk and root.

Another aspect of the data in table II pertains to the dynamic equilibrium within the leaf cells. Obviously the salts accumulated as a result of the wind were not largely used by the leaves in the construction of water insoluble compounds, for practically all the increased salt was water soluble. This is in harmony with the suggestion that the increased salt content was of value in protecting the leaves from excessive evaporation.

The conception commonly held regarding the ash content of leaves is that it increases permanently under certain conditions; but so far as we are aware this alternation of low and high salt content (the dynamic equilibrium concept of their inorganic constituents) has not before been emphasized or used in explaining physiological

troubles of leaves such as mottle-leaf. The ash content of citrus leaves subject to the action of winds may fluctuate considerably. During December, 1925, there were some warm but not excessively hot winds of very short duration. Samples of leaves were collected

TABLE III

RELATION OF ASH CONTENT OF CITRUS LEAVES TO ATMOSPHERIC CONDITIONS

| | | | DECEMBER 16, 1925 | DECEMBER 21, 1925 | DECEMBER 31, 1925 |
|---|---|---|---|---|---|
| Average wind velocity of preceding 3 days (miles per hour) | | | 9.1 | 4.9 | 4.1 |
| Relative humidity (percentage) | | | 50 | 69 | 49 |
| Mean temperature (° F.) | | | 50 | 47 | 57 |
| VARIETY | | ROOTSTOCK | ASH (PERCENTAGE DRY MATTER) | | |
| Navel orange | A* | Sweet orange | 15.04 | 14.70 | 15.24 |
| | A | Trifoliate orange | 14.81 | 14.99 | 17.63 |
| | B | Trifoliate orange | 15.71 | 15.83 | 15.81 |
| Valencia orange | A | Sweet orange | 18.34 | 17.79 | 18.67 |
| | B | Sweet orange | 16.62 | 15.23 | 16.25 |
| | A | Trifoliate orange | 17.40 | 16.74 | 17.35 |
| | B | Trifoliate orange | 17.19 | 16.94 | 16.83 |
| | A | Pomelo | 15.13 | 15.17 | 15.15 |
| | B | Pomelo | 17.61 | 14.26 | 15.15 |
| | A | Sour orange | 15.47 | 14.95 | 15.14 |
| | B | Sour orange | 15.77 | 15.80 | 18.36 |
| Average | | | 16.28 | 15.67 | 16.51 |
| Eureka lemon | A | Sweet orange | 16.61 | 13.95 | ..... |
| | B | Sweet orange | 17.35 | 16.19 | ..... |
| | A | Pomelo | 14.55 | 13.05 | ..... |
| | B | Pomelo | 17.19 | 15.33 | ..... |
| | A | Sour orange | 15.91 | 16.82 | ..... |
| | B | Sour orange | 19.79 | 17.91 | ..... |
| Average | | | 16.90 | 15.54 | ..... |

* In each case tree *A* stood at the north end of the row where it was fully exposed to the wind; tree *B* was the fifth tree southward and was therefore somewhat less exposed to the wind.

December 16, when moderate winds were blowing, and again on December 21 after a rather tranquil period. A third sample was collected December 31 when the wind again was blowing. The ash content of orange leaves collected on these three days is given in table III, with data on the ash content of lemon leaves on the first

two dates. The leaves in question were as nearly the same age as could be chosen, and successive samples were taken from the same trees. The Navel orange trees were somewhat protected on the north but the other trees were not. In each case sample *A* was collected from the tree on the north end of a row, and sample *B* from the fifth tree southward. It will be seen, however, that there were no very consistent differences between the two sets of samples. The data support the general conclusion of the former determinations, in showing that the ash content of the leaves increased during the period of desiccating winds and that it subsequently dropped. It will be remembered that the winds in December, 1925, were not severe, and the differences in ash content of the leaves is correspondingly less, in comparison with leaves collected in December, 1924.

The power of leaves to change their salt content bears little relation to the rootstock on which the tree was propagated. The ability of the leaves to increase their salt content may be looked upon as favorable to the tree, for it is only during the most severe ordeals that the leaves are lost with disastrous results. The stomata of mature citrus leaves do not actively function; hence if the salt content did not increase as it does, disastrous results would occur more frequently than they actually do.

The ash of fruits of Valencia oranges on the three dates mentioned showed no significant changes, the values found being 4.3, 4.21, and 4.0 per cent of the dry matter respectively. A further indication of constancy in composition was given by a determination of the ash of small fruits from the same trees on June 22, which showed ash amounting to 4.7 per cent of the dry matter. There would consequently be but little chance for the trees to withdraw inorganic constituents from fruits when a new set of leaves was being produced.

An increase in the ash content of the dry matter of mature citrus leaves may be brought about by a long period of warm weather with an inadequate supply of moisture in the soil. A remnant of a citrus nursery was abandoned in 1917 by the Citrus Experiment Station near the Rubidoux plots. Although the remaining trees were left without irrigation, some of them were still alive after about eight years. Early in the spring each year, the full sized leaves went into

a wilted condition upon the advent of hot weather, and remained in this condition until the beginning of the winter rains.

A sample of leaves was collected from the trees on July 29, after several weeks of hot weather. The surface of the soil around the trees was then converted into basins and filled with water to a depth of 3 inches. On the second, sixth, and twelfth days after irrigation other samples of leaves were collected and dried for analysis. Before irrigation the ash of the leaves was 15.52 per cent of the dry matter; two days after irrigation it was 14.39. The two subsequent samples showed very little further change.

TABLE IV

RELATION OF WIND INJURY TO AMOUNT OF INSOLUBLE NITROGENOUS MATERIAL IN MATURE ORANGE LEAVES

| DATE 1924 | VARIETY | ROOTSTOCK | TOTAL NITROGEN (PERCENTAGE DRY MATTER) | | | |
|---|---|---|---|---|---|---|
| | | | Insoluble | | Soluble | |
| | | | Injured | Uninjured | Injured | Uninjured |
| December 3.. | Navel orange | Sour orange | 2.08 | 1.83 | 1.17 | ..... |
| 3.. | Valencia orange | Trifoliate orange | 1.64 | 1.48 | ..... | ..... |
| 12.. | Valencia orange | Sour orange | 1.89 | 1.64 | ..... | 1.04 |
| 12.. | Valencia orange | Sour orange | 2.00 | 1.83 | 0.79 | 1.10 |
| 12.. | Valencia orange | Sour orange | 2.00 | 1.81 | 0.82 | 0.87 |

It is of interest that citrus leaves can live through such periods of prolonged and continuous wilting, and that upon irrigation they become turgid so rapidly and lose their increased salt content. A remark upon the difference between wilting and scorching seems appropriate. A wilted leaf will recover when adequate water is supplied from the shoot, but a scorched leaf never recovers. Wilting is not necessarily an intermediate stage of scorching. The blade of the scorched leaf may turn brown and become brittle in a few hours if exposed to desiccating wind. Its color is similar to that of a leaf which has been killed by immersion for a few minutes in boiling water. The behavior of a scorched leaf suggests that its death may be due to coagulation of the proteins of the protoplasm; in that case we might expect to find more insoluble nitrogen in the injured leaves.

Accordingly some of the material was analyzed for nitrogen. The dried material was extracted with water as stated, and the amounts found are shown in table IV. It is clear from the results that there was more insoluble nitrogen in the injured leaves, and it may therefore be assumed that the proteins were coagulated when the leaves were injured.

## Summary

1. The effect of desiccating winds upon citrus leaves was shown by their high transpiration and wilting. Analyses here reported show that there was a temporary accumulation of salts in the leaves accompanying excessive transpiration. The excess salt content of leaves which survived the windstorms disappeared during subsequent calm weather. There appeared to be an intimate relation between climatic conditions and salt content of leaves. The increased concentration of salt in the leaves appeared to be due largely to the increase in calcium.

2. The increase in salt content of the leaves was mainly due to an increase in the water soluble portion, especially that of calcium. It appeared that the increased salt content of the leaves was of value in protecting them from excessive evaporation, and emphasized the dynamic equilibrium between the leaf cell and its environment.

3. The loss of calcium through wind defoliation was inferred from the mottled condition of the next set of leaves, and the loss of organic nutrients such as carbohydrates and proteins was inferred from the small size of the subsequently formed leaves. In later cycles of growth the shoots produced healthy large leaves.

4. The larger amounts of insoluble nitrogen found in injured leaves made it probable that the desiccating wind had produced injury by coagulating the proteins of the leaf cells.

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# DIFFERENTIATION OF VASCULAR BUNDLE OF TRICHOSANTHES ANGUINA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 366

GRACE BARKLEY

(WITH PLATE VIII)

## Introduction

Notwithstanding the great progress of vascular anatomy during the past three decades, but little attention has been given to the development of the vascular bundle, although the development of the other parts of the plant has been known for some time. We are familiar with each step in spermatogenesis and oogenesis, from the archesporial cell to the reduction of chromosomes and the formation of eggs and sperms; but no such series has been described in the development of the vascular bundle, and still less is known of the origin and development of the various markings on the inside of the cell wall.

Morphologists have studied the embryo up to the differentiation of the dermatogen, periblem, and plerome; and vascular anatomists have studied the bundle after differentiation has taken place, but there is a region between the studies of the morphologists and the anatomists which seems to have been neglected by both. It was the original purpose of this investigation to study this neglected region in *Trichosanthes*, and some observations have been made along this line; but the material proved so favorable for a study of the origin of the markings on the spiral and annular vessels, and also of the origin of the bordered pits, that most of the investigation has been devoted to these features.

## Material and methods

*Trichosanthes anguina*, a tropical member of the Cucurbitaceae, seems favorable for a study of the differentiation of the bundle, since the phloem can be recognized in very early stages, and the long slender internodes make it possible to get very effective longitudinal

views. The material used was brought from near Calcutta, India, where it is native, to a station of the Bureau of Plant Industry at Miami, Florida. Seeds were secured from this station and planted in the University of Chicago greenhouse in September. The plants reached maturity late in the following summer, and began to die in October, a period of about thirteen months. The plant is a vine with a long slender angled stem. Specimens grown in the Chicago greenhouse in 6 inch pots reached a diameter of 1.3 cm. and a length of 8–16 m. At Miami, where it grows out of doors and rooted in the ground, it reaches a diameter of 2.2 cm., and, while the vine was not measured, the length was much greater than those in the greenhouse. In both cases the gourd was over 60 cm. in length and 5-7 cm. in diameter. The gourd is used for food in tropical countries, where the plant is widely cultivated for this purpose.

For topographical study, living and prepared sections were made from various places throughout the stem. The rest of the investigation was confined to the first thirteen nodes, where much of the differentiation takes place. The vine at this point seldom exceeds 2 mm. in diameter. The material was killed in a hot solution of mercuric chloride in 50 per cent alcohol with 5 per cent acetic acid. The stains used were combinations of light green and safranin, crystal violet and safranin, and iron haematoxylin. The safranin and light green stains were best for early vacuolization, crystal violet and safranin for the early stages in lignification, and the iron haematoxylin for some stages of the sieve plate.

## Topography

The mature stem has five angles and five deep furrows. A cross-section shows an epidermis with glandular hairs, some of which are capped with a single large cell, while others have a multicellular tip. There are also some with pointed tips. Hairs are not found on the older regions of the stem. The stomata are elevated.

Beneath the epidermis at the angles is a collenchyma tissue seven or eight cells thick. Beneath this and also beneath the epidermis between the angles is a layer of chlorenchyma about three cells thick. Next there is a layer of sclerenchyma about three cells thick in the young stem. As the stem grows older the sclerenchyma is found only

on the peripheral side of the bundles. A lysigenous cavity appears in the central part of the stem about 2 cm. from the tip, but it later becomes closed by the growth of the bundles. It is also found in the stem near the roots and in the petiole of the tendril.

There are ten bundles in the stem, five large ones occupying the angles and five small ones more deeply placed. The inner bundles grow more rapidly at first, and at a certain stage are much larger than the angle bundles. All the bundles are bicollateral, with the exception of two of the inner ones, and even these become bicollateral near the root. These two bundles are also peculiar in that they show no protoxylem except near the root.

There is fascicular cambium in the outer phloem. Cambial activity also produces lateral phloem in the older parts of the stem. This lateral phloem is not continuous along the side of the bundle, but appears here and there at the side of the xylem. One stem observed had a small bicollateral bundle produced by this cambium. Here the cambium was next to what corresponds to the inner phloem, and there was also cambium in the outer phloem of this bundle. Besides these patches of phloem there are independent patches here and there in the periphery.

The petiole usually has nine bundles, all of which are bicollateral except the two upper ones in the angles of the groove. The petiole of the tendril has five angles, in each of which is a bicollateral bundle. In the male peduncle there are seven to nine bicollateral bundles, but in the female peduncle there are as many as fifteen. There is great variation in the number of bundles in the petioles and peduncles, the number in the petiole varying from nine to eleven, in the tendril from five to seven, and the number in the male peduncle from seven to nine.

In tracing the differentiation of the bundle, it is very desirable to know the position of the young bundle in the topography, since the two bundles which are not bicollateral differentiate later than the others.

**Differentiation of bundle**

In tracing the development of the vascular bundle, it would be logical to begin with the undifferentiated meristem at the stem tip, and follow the gradual divergence of the meristematic cells until the

characteristic features of the mature bundle are attained; but practically, with the problem in its present condition, this method would be difficult and perhaps uncertain. Consequently the writer has adopted a method similar to that chosen by GREGOIRE when he began his series of investigations upon the structure of chromatin. Instead of beginning with the resting nucleus and tracing the chromatin through a continuous cycle, he began with the fully formed, easily recognizable chromosome and traced the changes which occur from that point on to the resting condition.

## PROTOXYLEM

The present study of the bundle begins at a stage in which the xylem and phloem portions are recognizable, but still more or less parenchymatous. A cell, which is to form a part of the spiral vessel of the protoxylem, will serve as a starting point from which changes will be noted as one proceeds toward the meristem and also toward the mature, spirally marked vessel.

About 3 mm. from the stem tip a conspicuous feature of the longitudinal section is one or more rows of large cells (fig. 1). These are parenchymatous and are very large compared with the adjacent parenchymatous cells. The lower cell is typical of this type. It has a length of 81.5 $\mu$ and a width of 9.8 $\mu$. The nucleus is also very large. Except in the size of the cell and nucleus, the cell is undifferentiated. Most of the interior of the cell is occupied with a large vacuole with protoplasm lining the cell wall, surrounding the nucleus, and connecting the latter with the peripheral cytoplasm. Toward the tip these cells become shorter and shorter, as is seen in the cells above the one described. They also become narrower, the nucleus smaller, the cytoplasm denser, and the cells similar to the neighboring ones in appearance.

In another cell (fig. 2) farther from the tip than this one, considerable differentiation has taken place. The nucleolus has disappeared and the outline of the nucleus is irregular, showing that it has begun to disintegrate. The central vacuole is very large, but strands of cytoplasm still remain, connecting the nucleus with the periphery. The peripheral cytoplasm has taken the spiral arrangement seen in the thickenings of the spiral vessel, with bands of more

finely vacuolated cytoplasm alternating with bands which are more coarsely vacuolate. A portion of this cytoplasm, more highly magnified, is shown in fig. 4, where the magnification is great enough to show the nature of the bands. They are composed of cytoplasm with vacuoles so small and numerous that the band looks homogeneous under a high power dry lens. Often there is only a single row of larger vacuoles between two bands, but sometimes there are two rows of larger vacuoles, as shown between the upper two bands of fig. 4. After the finely vacuolated portion of the spiral has become the thickened lignified band of the spiral, the protoplasm between the two rows of larger vacuoles appears as a line intermediate between the bands of the spiral (fig. 5 *l*). In an earlier stage than that of fig. 4 the cytoplasm between the rows of vacuoles appears as faint granular striations which are seen only with careful focusing (fig. 3). At first sight these striations look like rows of granules, but they are the result of regularly placed vacuoles, and the apparent granules are the denser portions of cytoplasm between them. Unless well fixed and stained, the cytoplasm at this stage looks like that shown in fig. 1, where the arrangement into bands has not yet begun. The faint striations (fig. 3) become the finely vacuolated bands (fig. 4), which later become the lignified bands of the spiral thickening (fig. 5).

In fig. 6 the length of the individual cells that contributed to the formation of the spiral vessel can be seen because the end walls are not yet completely absorbed. The length of the middle cell in this figure is 97.8$\mu$. While the spiral band in this cell is less reddened with safranin than in the vessel below, showing less lignification, the band is almost as large in cross-section, and the cell itself has the same diameter as the cell below. It seems safe to conclude that the differentiation is only in the degree of lignification.

While the cell just above this one is the next adjacent cell, it is considerably less differentiated. The spiral band is definitely outlined but is not at all lignified. Vacuoles are still present, the spiral band is quite granular in appearance, and, although much thicker than the cytoplasmic band in fig. 2, it is not so much thickened as in the cell below.

The three cells in this row, in which the end walls have not broken down, differed widely in the differentiation of the spiral. A strik-

ing feature of the development of the spiral at this stage is that there is no gradual advance in passing from one cell to the next, but a sharp difference in the degree of differentiation between adjacent cells. Later, when the transverse walls have broken down, the development of the spiral becomes uniform throughout the vessel. That these stages are passed through very quickly is shown by the fact that they are seldom found, but when they are seen they always present this sequence of development.

There are three cells between *a* of fig. 6 and the parenchymatous cells of the type of fig. 1, near the meristem end of the row. The third cell above *a*, adjacent to the parenchymatous cells, while much less differentiated (having flatter bands with more vacuoles than the two cells between it and cell *a*) is more differentiated than the cell in fig. 2, for its nucleus has broken down and disappeared.

The annular vessels are formed from cells much longer and narrower than those which contribute to the formation of the spiral vessel. The cell in fig. 7 has a length of 166.6 $\mu$ and a width of 7.8 $\mu$. This cell also has a very long nucleus with a length of 26.9 $\mu$ and a width of only 4.3 $\mu$. The nucleus has several nucleoli and the outline is even, showing that the nucleus has not yet begun to disintegrate. There is here a large central vacuole occupying most of the cell, but cytoplasmic strands still connect the nucleus with the peripheral cytoplasm.

The peripheral cytoplasm, instead of having rows of small vacuoles, as seen in the young spiral vessel, has larger vacuoles extending entirely across the cell. In the upper part of the cell there is no definite arrangement of cytoplasm showing any differentiation, but in the lower part there are large vacuoles with bands of cytoplasm between them. The cytoplasm is denser where it borders upon the larger vacuoles, which, being close together, crowd the denser layers of cytoplasm together, thus forming bands. Above the nucleus is a single band of cytoplasm. Just below this cell is one which is well differentiated, with a series of short loose spirals and rings. Although not yet completely lignified, the cross area of the thickenings is about as great as in the mature vessel.

The protoxylem has several kinds of vessels, the annular, loose spiral, and close spiral. While the annular vessel, just described, was

first differentiated with the formation of rings, after the close spiral near it was quite well differentiated and partially lignified, it reaches complete differentiation sooner than the spiral vessel. At that level of the stem where the annular vessel first seems completely lignified, the spiral thickenings are still stained purple with crystal violet and not nearly so red with safranin, showing that they do not lignify as rapidly as the thickenings of the annular vessel. The thickenings of the latter also have a greater cross area at this time.

Some of the thickenings of the young close spiral have a faint line between the spirals, as if a deposit of the last remaining vacuoles had been left between the main coils. These faint lines doubtless are due to the weak band of cytoplasm between two rows of vacuoles, as already described and illustrated in figs. 4 and 5. Many of the thickenings have a clear streak through the center before complete differentiation, giving the thickening a double appearance. After differentiation these coils are heart-shaped in cross-section, and in some there is even a complete separation of the two parts for short distances, making a slit in the spiral. Some of the loose spiral vessels have two coils in parts of the vessel, each coil being about as far distant as the coils of a single loose spiral vessel. Perhaps this was caused by the same process, only proceeding further so as to make two separate coils. The thickenings of the mature vessel have two regions in cross area, a central area surrounded by an outer border. The outer area seems to have the greater lignin content.

When a spiral protoxylem vessel is adjacent to a pitted metaxylem one, about the time the metaxylem begins to pit, several small vacuoles become visible between the coils of the spiral (fig. 5). These enlarge and lengthen and take a definite arrangement. They become the elongated pits between the coils of the spiral. In its younger stages this pit is occupied by a row of small vacuoles.

### Metaxylem

Although the protoxylem is the first element of the bundle to differentiate, the metaxylem is far more conspicuous. Even after the protoxylem vessels are lignified, the metaxylem cells, although large and still elongating, are thin walled, without any traces of the markings which characterize the mature vessel. They present a greater va-

riety of form than is found in the protoxylem. In the transverse section of the young bundle there are several cells of very large lumen, and these are the large cells of the metaxylem. In longitudinal section the diameter of these cells exceeds the length. Other cells of the metaxylem with smaller diameter are very much longer, but by breaking down of transverse walls both types develop into vessels. Beside these there are smaller parenchyma cells which surround the vessels. Those parenchyma cells surrounding the larger vessels are irregular and sinuous in outline, and those surrounding the smaller vessels have long straight walls.

The first cells of the metaxylem to differentiate are not those of the largest diameter, but the long cells of considerably less diameter. In these the markings are scalariform, with pits so long that in some places they give the impression of a spiral. This cell was formed under conditions more nearly like those which prevailed while the protoxylem was being differentiated, and the markings naturally more nearly resemble those of the protoxylem than do the markings of the other metaxylem vessels which are laid down later.

The next cell to differentiate is intermediate in size between the scalariform cells and the cells of the largest lumen. This intermediate type is comparatively short, the length being only two or three times the diameter in some cells; but there are some cells of this type with a length seven or eight times the diameter. These have long pits, although not so long as those of the scalariform vessel. The last of the vessels to differentiate is the one formed by the large cells mentioned, which are still thin walled when the walls of the other vessels have thickened.

The character of the markings on the metaxylem is different from that on the protoxylem, but the process of formation is very much the same, and the difference is probably due to the different conditions under which the thickenings are laid down.

The scalariform vessel resembles the spiral so closely in its markings that it seems safe to conclude that the thickenings were formed in almost the same way. The thickened band is a little broader and flatter. The pit spaces in early stages are filled with tiny vacuoles, giving the same granular appearance that was found between the coils during the early stages in the formation of the spiral.

The next cell to differentiate bears a similar resemblance to the scalariform type that the scalariform one does to the spiral. Probably the resemblance is due to the fact that two types are formed under somewhat similar conditions. In the same way this last vessel to differentiate would resemble the scalariform, because the conditions which prevail during the formation of the markings are somewhat similar. The thickening is quite evenly distributed over all parts except the pits. These are elongated and in some cells they are so long as to give the cell a scalariform appearance. The pits are formed and the walls are quite thick before the nucleus begins to disintegrate. The lateral walls are slightly lignified when the end walls break down.

The pits of the largest cells vary in shape from elongated to round, and are closely crowded, while the pits of the surrounding parenchyma are small and slightly elongated.

The vacuoles of the young cells of the metaxylem do not have as definite an arrangement as they had in the protoxylem vessels, but they are arranged somewhat in such horizontal rows as are found in the elongated pit markings. These, however, are not as definite in arrangement as in the protoxylem. According to STOVER,[1] the first thickening of the desmogen strand is laid down in pitted form, and these vacuoles cause the cytoplasm to line the wall in the same pattern as the first thickenings. So far as the pitting of the metaxylem is concerned, the writer's observations agree with those of STOVER; but as to the origin of the spiral and annular markings of the protoxylem, the development in *Trichosanthes* differs from his account. When the pits are first formed they are shallow and not at all bordered, but as more and more material is deposited in thickening, it overarches the original pit and forms the border. This is the origin of the bordered pit in *T. anguina*, and may be true for bordered pits in general.

## PHLOEM

It has long been known that the sieve tube and companion cell of the Cucurbitaceae are very large. In *Trichosanthes anguina* these elements are not only large but are differentiated very early, so that it is very easy to distinguish the xylem and phloem elements in a

[1] STOVER, E. L., The anatomy of *Calamovilfa*. Ohio Jour. Sci. **24**: 169–178. 1924.

young bundle. The companion cell is cut off from the sieve cell even before the metaxylem begins to show any markings.

The sieve tube in the mature stem is very long and narrow, with a length varying 150–700 μ and a width of 17-30 μ. The tube continues to lengthen long after it is differentiated. The sieve cell can be recognized with certainty within 1.5 mm. from the stem tip, for at this distance from the tip it has cut off the companion cell, and perforations can be distinguished in the sieve plate (fig. 9). The nuclei persist until the sieve plate reaches its mature condition. In the early stage mentioned, the shortest cell in the figure has a length of 12 μ and the longest a length of 27 μ. The protoxylem at this level is differentiated with markings, but they are not lignified and the metaxylem shows no traces of markings.

The sieve character of the sieve plate can be recognized even before the companion cell is cut off. This is proved by the fact that the perforations can be seen in young stages between adjacent companion cells. On the sieve tube portion the plate thickens unevenly, the thickening being laid down faster on the side toward the root. The thickening process continues for a long time and does not reach its maximum until the nucleus has disappeared.

The early formation of the companion cell makes it easy to recognize the phloem region. The companion cells do not lengthen enough to keep pace with the sieve tube but divide transversely, making a row of companion cells for each sieve tube. They are filled with a very dense cytoplasm containing many large vacuoles.

Since eight of the ten bundles are bicollateral, the stem has a large proportion of phloem. Lateral phloem containing sieve tubes is also found at the sides of the xylem in the older parts of the stem. The phloem is composed of sieve tubes, companion cells, and parenchyma cells, the latter being long and slender.

## Cambium

The cambium is not a persistent part of the desmogen strand, but is a new formation arising between the xylem and phloem, just as phellogen arises in the cortex (fig. 12). The cambium does not appear until the xylem and phloem are easily recognizable, as shown in fig. 12. The cells of the cambium have a denser protoplasm than

that of the surrounding cells, and are considerably elongated. Both the cambium cells and the phloem parenchyma have their end walls very little inclined.

Cambium is found between the xylem and the outer phloem, and in a very small amount between the xylem and the inner phloem. It is also found along the side of the xylem of the bundle in the older parts of the stem. This lateral cambium produces a phloem, and even a small bundle with both xylem and phloem was produced by this cambium in one specimen observed. Traces of interfascicular cambium are found near the nodes in stems about 3 mm. in diameter, but there is no interfascicular cambium in the other parts of the internode.

There are patches of tissue in the peripheral parts of the older stem where meristematic activity sets up. This may be because the stresses upon *Trichosanthes*, as a climbing vine, cause traumatic activity to set up as a traumatic response.

## Summary

1. *Trichosanthes anguina* is favorable for a study of the differentiation of the vascular bundle, because the phloem can be recognized very early and the long internodes make it easy to get straight views of the differentiating structures.

2. The spiral vessel of the protoxylem in its early stages has bands of peripheral cytoplasm which precede the spiral markings and have the same arrangement, and become the basis of the lignified spiral. The position of the cytoplasmic bands is determined by rows of vacuoles in the cytoplasm immediately preceding and during the formation of the cytoplasmic bands. The process of the early differentiation of the marking is a very rapid one. The position of the bands of the annular vessel is also determined by vacuoles, but the determining vacuoles are much larger than those of the spiral vessel.

3. The young cell of the metaxylem also has vacuolated peripheral cytoplasm which precedes the formation of its markings. The scalariform vessel, composed of long cells, is the first to differentiate; the vessel composed of shorter cells with elongated pits is the next to differentiate; and the vessel composed of short cells with large lumen marked by round or oval bordered pits is the last to differentiate.

4. The sieve tube differentiates very early. It cuts off the companion cell after the perforations have been formed in the sieve plate. The companion cell may divide transversely several times, so that there may be as many as six or seven along a single sieve cell. The callus of the sieve plate is much thicker on the side next to the root.

5. The cambium does not appear until the xylem and phloem are easily recognizable, and there is scarcely any interfascicular cambium, except near the nodes. Lateral phloem is derived from a cambium which appears still later.

The writer wishes to express appreciation to Professor C. J. CHAMBERLAIN, under whose direction this investigation was carried on.

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## EXPLANATION OF PLATE VIII

FIG. 1.—Cell row which will contribute to formation of spiral vessel; ×640.

FIG. 2.—Arrangement of vacuoles in cytoplasm in early formation of spirals, *s*, coils of young spiral; ×1330.

FIG. 3.—Earlier stage than that shown in fig. 2; ×1330.

FIG. 4.—Portion of cytoplasm in fig. 2, enlarged from camera drawing, showing nature of spiral bands; ×5320.

FIG. 5.—Vacuoles between coils of spiral vessel in early stages of pit formation; ×1330.

FIG. 6.—Cells of spiral vessel before end walls are absorbed, showing difference in differentiation; ×580.

FIG. 7.—Cell contributing to formation of annular vessel, showing early stage in formation of ring; ×1330.

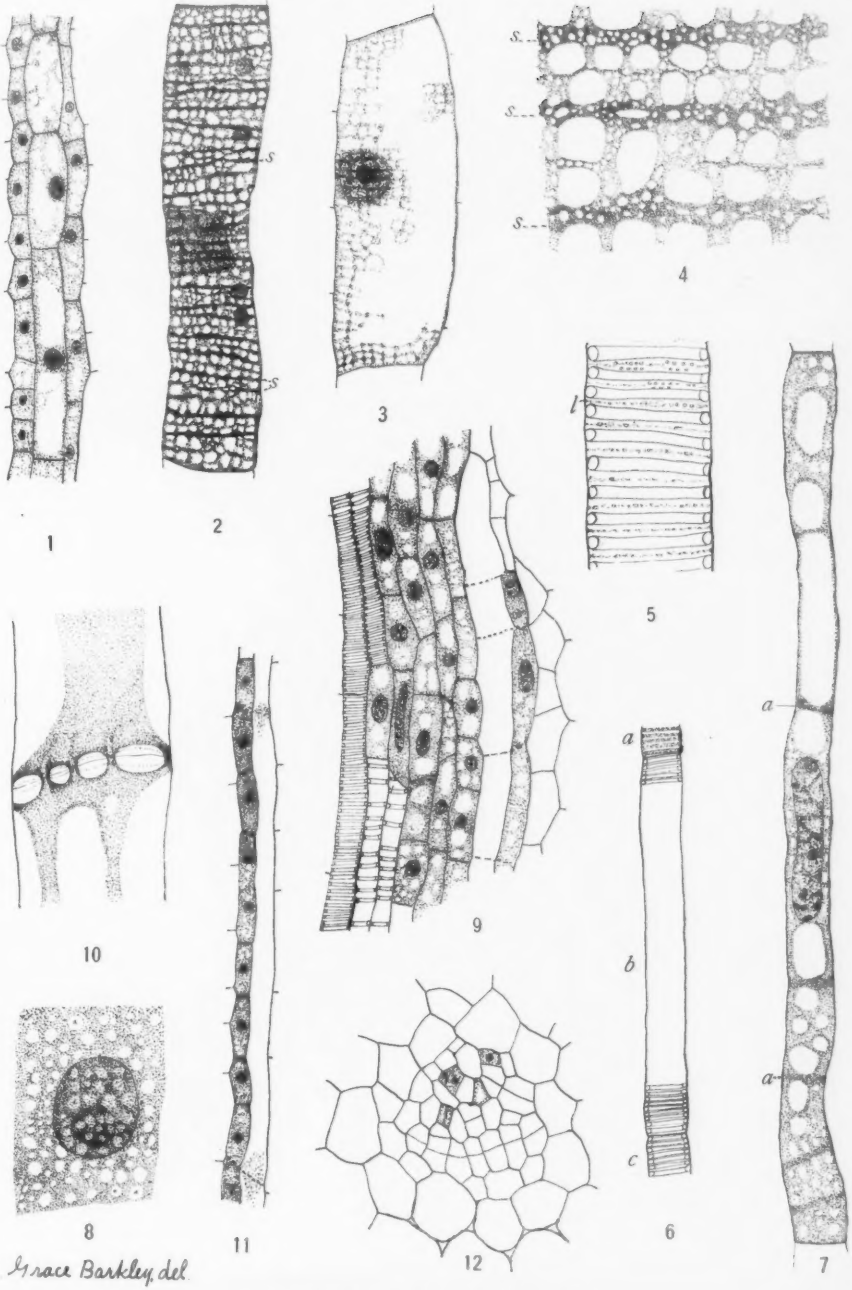
FIG. 8.—Vacuoles in peripheral cytoplasm of young metaxylem cell; ×1330.

FIG. 9.—Young sieve cell and companion cells, showing sieve plate extending between adjacent companion cells; ×640.

FIG. 10.—Sieve plate showing greater thickening on side toward root; ×1330.

FIG. 11.—Sieve tube with row of companion cells; ×580.

FIG. 12.—Origin of cambium in young bundle after other elements are recognizable; ×340.




BARKLEY on TRICHOSANTHES

# DISTRIBUTION AND ABUNDANCE OF SPARTINA MICHAUXIANA AT DOUGLAS LAKE, CHEBOYGAN COUNTY, MICHIGAN[1]

F. C. GATES, EDITH C. WOOLLETT, AND E. P. BREAKEY

(WITH TWO FIGURES)

## Introduction

From the standpoint of rapidity of spread in a new habitat in the Douglas Lake region, one of the most interesting plants has been *Spartina Michauxiana* Hitchcock. The activity of this plant, which is nominally a prairie species, had been observed for several years when the appearance of OLIVER'S [2] paper on the colonization of mud flats in southern England and northern France by *S. Townsendii* made it seem rather worth while to record the facts in regard to *S. Michauxiana*.

Just exactly when *Spartina* first made its appearance in the Douglas Lake region is not known, but old lumbermen say that it was not present anywhere at the time the region was lumbered in the 188o's. Presumably it made its appearance in the early years of the present century, possibly at the time when a grade was established for a lumber railway. At any rate, when first observed it was present only in a very few places along this grade and the southwest corner of Douglas Lake, where the grade runs but a short distance from the lake. It has now spread to nearly all parts of the shore of the lake in varying abundance. Since being under observation, for a time *Spartina* extended its patches along the grade, but when the grade was abandoned as a road, the encroachment of the forest with its attendant shade eliminated the plant there. Along the lake, however, it has been spreading vegetatively from year to year in a rather remarkable way. In different years the colonization of new places has

[1] Contribution no. 261 from the botanical laboratory of Kansas State Agricultural College, and a contribution from the Biological Station of University of Michigan.

[2] OLIVER, F. W., *Spartina Townsendii;* its mode of establishment, economic uses and taxonomic status. Jour. Ecol. 13:74-91. 1925.

been studied, particularly in the Sedge Point region. The plant has made headway against all strand types of native vegetation, but is limited in advancing far from the lake by any vegetation that is capable of shading it. The present paper is the result of the detailed study

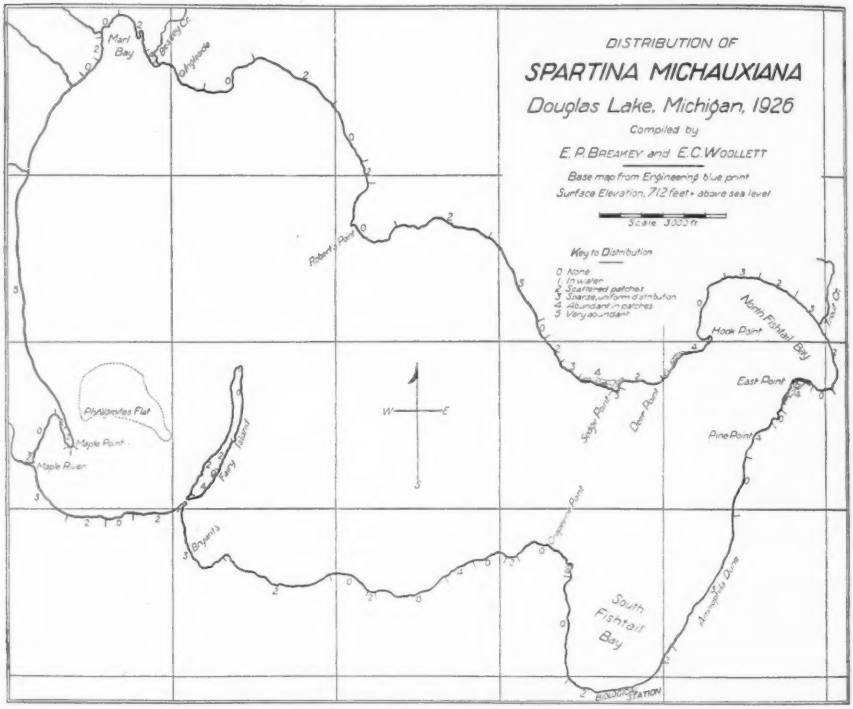



FIG. 1.—Map showing distribution of *Spartina Michauxiana* at Douglas Lake, Michigan, 1926; by E. P. BREAKEY and E. C. WOOLLETT.

of the distribution and characteristics of the plant made especially by the junior authors during the summer of 1926.

Douglas Lake is one of several larger inland lakes lying in the Cheboygan River drainage system at the northern end of the lower peninsula of Michigan. Douglas Lake lies entirely in glacial deposits, but it seems certain that the depression existed prior to the last retreat of the glacier, which filled it with small lobes of ice that, melting

more slowly than the main ice sheet, prevented heavy deposition.[3] The land surrounding the lake is all of glacial origin, and the shore line is composed primarily of sand with brief stony stretches. With a surface area of 14.92 sq. km. the lake has a perimeter of 24.97 km. Prevailing winds for the region are from the northwest, except for the months of September, October, and December, when the direction is from the southwest. The main axis of the lake is approximately northwest-southeast, with a total length of about 7 km. The $P_H$ reading of the surface waters of the lake varies but slightly from 8.6. The annual precipitation is about 73 cm.[4] The entire region lies in the transition area between the northeastern coniferous forests and the deciduous forests of the south. The shore of the lake is narrow, with small projections and indentations, especially along the south. Small dunal areas, especially the *Ammophila* dune (fig. 1), are present where the prevailing winds have piled the sand, and vegetation has become established. At various points around the lake small bluffs arise. The shore may be divided into shoal, lower, middle, and upper beaches, and upland vegetation areas. In protected coves, where conditions are favorable for suitable development and growth, the associations of the beach inland from the water include the *Scirpus validus*, *S. americanus*, *Eleocharis palustris*, and *Iris* associations, and the invading *Spartina Michauxiana* meadows.

### Present distribution

The distribution of *Spartina Michauxiana* on the shores of Douglas Lake ranges from scattered isolated bunches of a few plants to rather large meadow-like zones, mostly on the lake shore at the edge of the upper beach, although in two places on the lower beach it was found growing out actually into the water. At Maple Point plants were growing in the water, but not where water would be expected to cover them all the year. A layer of aerenchymatous tissue was present in the anchorage roots, but not in the rhizomes of those plants growing in the water. The rhizomes adapt themselves to an

[3] SCOTT, I. D., Inland lakes of Michigan. Mich. Geol. and Biol. Survey, Publ. 30. Geology Series 25. 1920.

[4] For the detailed figures of meteorology of the Biological Station, see GATES, F. C., Meteorological data, Douglas Lake, Michigan. Mich. Acad. Sci. Arts, and Letters 4:475-489. 1924; and other papers referred to there.

average depth of about 8 cm. If they are buried they grow up to meet this level; if the sand is removed from around them they grow down to the level. At no point in the survey were seedling plants found, nor have they been noticed in previous years, apparently indicating that reproduction here is entirely vegetative. On the shore west of Bryant's hotel near the end of Fairy Island, five of the plants were found on the lower beach near the water's edge. Everything would indicate that these plants had recently been torn loose from some section of the lake shore to the northwest, and had been carried to this spot by the wind and waves and cast upon the beach. Three of the plants were well established near the water's edge. The roots of a fourth plant had been covered with sand by the waves, but it still remained in a leaning position. The fifth plant lay on the beach at the water's edge, with only a few of its roots covered. This indicates a method by which the plant is distributed around the lake shore. On Deer Point, where *S. Michauxiana* is well established, it was also found growing out into the water, invading the *Scirpus americanus* association. As 1925 was a year of low water and in all probability the *Spartina* was not then covered, it remains for the future to decide whether or not the water will be an eliminating factor. In the vicinity of Maple River in sloughlike places *Spartina* is entirely absent, although *Calamagrostis canadensis* and *C. inexpansa* are well established. In contrast with the nearly pure sand of the lake shore, the soil near Maple River is of a peatlike character.

Upon the lower and upper beaches, especially along the shore between Marl Bay and Maple Point, *Spartina* has invaded the *Eleocharis palustris* and *Iris* associations, and has established itself in abundance. In several places in this area *Myrica Gale* has shaded out the *Spartina* at the foot of the ice ridge, but on the ridge itself beyond the shaded area it still is growing. In other spots *Spartina* meadows of varying width were found competing with *Scirpus americanus* and proceeding toward the water's edge. In clearings near Bryant's hotel isolated stands are invading the *Scirpus americanus* and *Potentilla Anserina*, and the *Spartina* would be well established were it not for human interference. At Sedge Point three beach pools have been formed by sand bars, thrown across the point by the winds which blow along the shore. On the small sand dune skirting the

pools *Spartina* is quite abundant and is advancing toward the pools, competing with the *Iris* association. On the small dune behind the pools *Spartina* is somewhat well established, and has displaced the native *Iris* association. On the broad marly shoals found between Robert's Point and Bessey Creek, that are subjected regularly to very severe ice work and wave action, it is difficult for the *Spartina* to hold its own in competition with the *Scirpus* and *Eleocharis* associations.

The amount of *Spartina* on Maple Point is probably lessened because of severe ice action, as only scattered patches of the plant were found, although vigorous growth for the year was at its optimum. Ice work has cut back both the *Spartina* and *Scirpus* on Deer Point, which is not too well protected. Between Sedge Point and Bessey Creek the distribution of *Spartina* is very sparse and scattering, due to the severe ice and wave action to which this area is regularly subjected. This is shown by the fact that in the vicinity of Robert's Point numerous specimens of *Acer rubrum* and *Populus balsamifera* have been undercut and overturned by ice and wave action. In this same strip of shore, ice has cut away whole shoal associations, leaving only a brown carpet of matted roots and rhizomes. At East Point the *Spartina* is located primarily upon the dune fringing the northern side of three small beach pools, formed by winds blowing along the shore and offshore winds. Very severe ice action is noticeable at this point on the northwestern shore. In the area between Bryant's hotel and the Biological Station there is a long stretch of stony shore, where water-washed rocks and gravel with the help of ice and wave action keep *Spartina* from developing. Five good patches with sand areas between show that, provided eliminating factors became less severe in action, the *Spartina* would probably advance rapidly. The north and east winds are the more important on this shore, because of the protection from the westerlies offered by Fairy Island.

Shade is an eliminating factor that is especially noticeable in the cove to the north from Hook Point, and the eastern side of Grapevine Point. In such places *Spartina* is wholly absent. In protected coves where the water is shallow and wave and severe ice action are minimum factors of elimination, the plant is well established provided shade is not an eliminating factor. A survey of Fairy Island

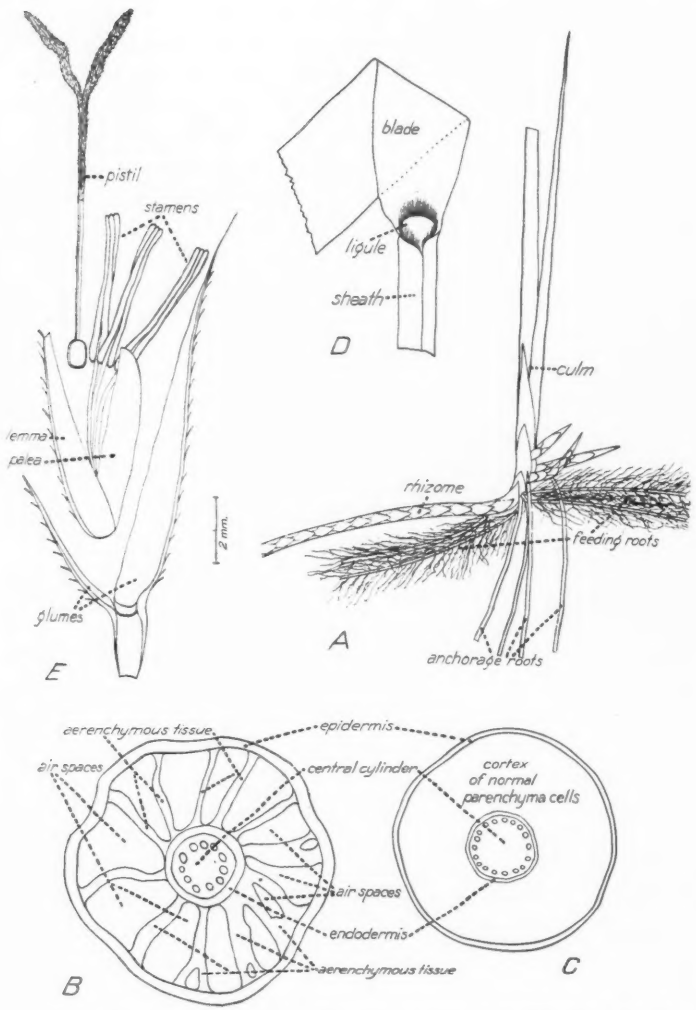



FIG. 2.—Drawings of *Spartina Michauxiana* showing: A, basal part of plant, ×0.5; B, C, diagrammatic cross-sections of anchorage roots (B) and ordinary roots (C); D, detail of part of leaf enlarged; E, details of spikelet (scale shown); by E. P. BREAKEY.

showed that on the eastern shore shade is the principal eliminating factor, with ice action as secondary. Where sufficient sunlight is afforded in this area large patches of *Spartina* are found. In several places near Maple Point where *Myrica Gale* has grown out to the water *Spartina* has been shaded out.

As a dune holder *Spartina* compares well with other plants at Douglas Lake. Its ability to hold sand is best shown at Sedge Point, where a permanent *Spartina* dune is developing. *Ammophila arenaria* is the best dune holder in the region, but is of little importance at Douglas Lake because of the lack of suitable conditions for its dune-developing capacities. Upon the one *Ammophila* dune at Douglas Lake *Spartina* and *Elymus canadensis* are the only grasses competing with *Ammophila*. *Spartina* is becoming very well established on this dune, which has an average height of 2 m. above the level of the lake. At various places, especially along the eastern shore of the lake, *Spartina* is holding small seasonal dunes. The structure of the plant appears to be well adapted to living in shifting sand provided it is not too high. The ability of the rhizomes to adjust themselves to changing levels fits *Spartina* for such a habitat, and in this region its economic importance is primarily that of a dune holder.

Late in June 1926 several sods of *Spartina* were removed from their habitat on the lower beach and transplanted upon Phragmites Flat, in about 12-15 cm. of water. The flat is a broad gravel bar in the lake and shows above water only during low water years. Up to the time of the transplanting of *Spartina* upon the flat, *Phragmites communis* was the only grass established there, where it followed the natural depression around the southern edge. At the end of the summer the flat was again visited to determine how the *Spartina* had fared in its new habitat. The plant had become established during the summer and new shoots were pushing up from rhizomes growing out through the sand and gravel under water.

On the whole, although *Spartina Michauxiana* is well established in certain areas around Douglas Lake, the eliminating factors of shade, ice, and wave action appear to control the distribution at all points.

## Systematic study

A systematic study of the plant itself was made: Culms 72-(122)-143 cm. high. Leaves 2.8-(5.5)-9.1 dm. long, tapering to a very slender point, keeled, flat, but quickly involute in drying, smooth except the margins. Spikes 10-(14)-18, scattered, spreading, 4-7 cm. long, rachis rough on margins; glumes serrulate-hispid on keel, the first finely hispid and serrulate on inner margin, acuminate, and shorter than the floret; the second finely hispid and tapering into a serrulate-hispid awn 2-5 mm. long; lemma 7-9 mm. long, finely hispid and with a serrulate scabrous midnerve which abruptly terminates below the emarginate or two-toothed apex.

Comparing this with the description given in GRAY'S manual brings out the fact that the *Spartina Michauxiana* in the Douglas Lake region is in all respects a smaller plant, less rank, with the first scale shorter than instead of equaling the floret, the awn decidedly shorter, and with the glumes and lemma, except for the keel, finely hispid instead of glabrous. Studies later at the Herbarium of the Field Museum of Natural History, and field studies in northeastern Illinois and eastern Kansas tend to confirm the impression. HITCHCOCK [5] states, however, that the description in the manual was too closely drawn, and does not give sufficient leeway to the variation exhibited in specimens, so that it is perhaps best to consider the Douglas Lake plants (and also specimens from lakes in northern Wisconsin and Minnesota) as an ecological variation or ecolog (arenaria) of a prairie plant in a northward or northeastward advance upon sandy shores of northern lakes, with perhaps a further suggestion of the possibility of having a new species in the process of evolution, thus meriting further attention.

## Summary

1. The distribution of *Spartina Michauxiana*, nominally a prairie plant, on the shores of Douglas Lake, Cheboygan County, Michigan, ranges from scattered isolated groups of a few plants to rather large meadow-like zones.

2. The habitat of the plant is that afforded by the lake shore, where it grows typically on the upper beach in front of the upland

[5] In a personal letter to the senior author, after examining a series of specimens from the Douglas Lake region.

vegetation, although in two places it was found on the lower beach growing out actually into the water.

3. The eliminating factors of shade, ice, and wave action seem to control the distribution at all points.

4. *Spartina* is of economic importance in the region primarily as a dune holder in situations not too far above the lake level.

5. The plants of *Spartina Michauxiana* in the Douglas Lake region, although they differ in several particulars from the standard descriptions, at present favor suitable extensions of the description to cover these plants as ecological variations rather than consideration as a new species.

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# FURTHER STUDIES ON GROWTH OF CHLORELLA AS AFFECTED BY HYDROGEN-ION CONCENTRATION

## ALKALINE LIMIT FOR GROWTH

F. B. WANN AND E. F. HOPKINS[1]

(WITH THREE FIGURES)

In earlier papers (1, 2) it was shown that in a highly buffered culture solution containing calcium, the true effect of the H-ion concentration on the growth of *Chlorella* sp. was masked by the lack of available iron in the more alkaline cultures. This condition was produced not alone by chemical precipitation of the iron, but largely also by its adsorption on the amorphous precipitate of calcium phosphate present in all the solutions more alkaline than $P_H$ 5.7. It was further shown that, while the presence of sodium citrate would prevent the chemical precipitation of iron at all H-ion concentrations where this otherwise occurs, it did not hinder the adsorption of iron on the calcium phosphate precipitate which forms in culture solutions containing calcium at alkaline reactions. By omitting calcium the adsorption of the iron was prevented, while the addition of sodium citrate to the medium served to maintain the iron in solution in cultures as alkaline as $P_H$ 7.5. In such a modified culture solution maximum growth of *Chlorella*, as measured by the dry weight of the crop produced, appeared at $P_H$ 7.5, the most alkaline solution of this series. The present paper presents the results of experiments with solutions of H-ion concentrations ranging from $P_H$ 5.0 to 9.5, the purpose of the investigation being to establish if possible the alkaline limit for the growth of *Chlorella*.

### Methods

The procedure adopted was the same as that previously reported. The culture medium consisted of equal portions of two solutions,

[1] Continuation of cooperative experiments begun at Cornell University under fellowships in the Biological Sciences, National Research Council. The writers wish to express their appreciation to both of these institutions for facilities which made the investigation possible.

designated Solution A and Solution B, which were combined after sterilization. Solution A contained 1.0 gm. $NH_4NO_3$, 0.4 gm. $MgSO_4.7H_2O$, 0.4 gm. sodium citrate, 0.8 mg. ferric iron, and 20 gm. glucose per liter. The iron was supplied from a standard solution of iron wire in dilute hydrochloric acid. Solution A was introduced in 25 cc. quantities into a number of Pyrex Erlenmeyer flasks, and these were immediately sterilized. Solution B consisted of eleven different phosphate buffer mixtures prepared from M/7.5 concentrations of $K_2HPO_4$, $KH_2PO_4$, and KOH, the mixtures being selected to give H-ion concentrations ranging from $P_H$ 5.0 to 9.5. Since earlier experiments had established rather definitely the shape of the $P_H$ growth curve on the acid side of $P_H$ 5.0, solutions more acid than this were omitted from the present series of cultures. The buffer mixtures were prepared in lots of 100 cc. each and then introduced into Pyrex Erlenmeyer flasks in 25 cc. quantities so that four replications of each $P_H$ were provided. After sterilization the buffer mixtures were combined with Solution A. It should be noted that by the combination of Solutions A and B the concentrations recorded for these two solutions were reduced one-half in the final culture medium. Of the four replicates of each $P_H$, three were inoculated, the remaining one being used for determinations of the initial $P_H$ and initial iron content. GILLESPIE'S colorimetric method was used for all the H-ion concentration determinations, while the iron content was determined by the method of MARRIOTT and WOLF as modified by the authors (1). The inoculations were made from a uniform suspension of *Chlorella* sp. cells in 0.6 per cent NaCl, 0.5 cc. being used for each culture. The cells were obtained from a pure culture of the organism on agar slants. The usual aseptic precautions were observed throughout.

### First experiment

After an interval of two weeks, during which the cultures were maintained in the greenhouse under partial shade, determinations were made of the dry weights of the crops produced and of the final H-ion concentrations of the culture solutions. Tests for soluble iron were also made on some of these solutions. The clear culture solution was obtained for these tests by centrifuging the contents of each

culture flask and decanting the supernatant liquid into large Pyrex test tubes. The algal cells were then washed into Gooch crucibles containing asbestos mats, and the dry weight of each crop ascertained after drying at 100° C. for 18 hours. The data obtained from the first experiment are presented in table I.

The tests for initial iron content showed as much soluble iron present at $P_H$ 8.5 as at $P_H$ 5.1, while at $P_H$ 9.4 the amount appeared to fall slightly below that of the other solutions tested. The actual

TABLE I

DATA FROM FIRST EXPERIMENT

| CULTURE NO. | $P_H$ INITIAL | INITIAL FE PER CULTURE (MG.) | $P_H$ FINAL | | | FINAL FE PER CULTURE (MG.) | DRY WEIGHT OF CROP (MG.) | | | AVERAGE CROP (MG.) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | | A | B | C | |
| 1..... | 5.1 | 0.0475 | 4.6 | 4.5 | 4.6 | 0.023 | 71.0 | 81.1 | 76.6 | 76.2±1.97* |
| 2..... | 5.8 | 0 | 5.5 | 5.5 | 5.5 | 0 | 72.4 | 83.8 | 77.6 | 77.9±2.20 |
| 3..... | 6.4 | 0 | 6.1 | 6.0 | 6.2 | 0 | 71.4 | 88.5 | 79.8 | 79.9±2.34 |
| 4..... | 6.9 | 0 | 6.6 | 6.7 | † | 0 | 77.9 | 81.8 | † | 79.8±1.31 |
| 5..... | 7.2 | 0 | 7.2 | 7.2 | 7.2 | 0 | 60.1 | 55.8 | 65.4 | 60.4±1.87 |
| 6..... | 7.6 | 0.0475 | 7.3 | 7.4 | 7.4 | 0.032 | 58.9 | 48.7 | 58.6 | 55.4±2.26 |
| 7..... | 7.8 | 0 | 7.6 | 7.6 | 7.5 | 0.046 | 29.0 | 42.3 | 46.9 | 39.4±3.61 |
| 8..... | 8.0 | 0.0475 | 7.7 | 8.0 | 7.9 | 0.032 | 18.1 | 04.6 | 07.4 | 10.0±2.73 |
| 9..... | 8.5 | 0.0475 | 0 | 0 | 0 | 0.037 | 0 | 0 | 0 | 0 |
| 10..... | 8.9 | 0 | 0 | 0 | 0 | 0.037 | 0 | 0 | 0 | 0 |
| 11..... | 9.4 | 0.0375 | 0 | 0 | 0 | 0.037 | 0 | 0 | 0 | 0 |

0, no determination made.

* Probable error determined by BESSEL'S formula.

† Culture contaminated.

amount of iron added to the original solution was equivalent to 0.02 mg. ferric iron per culture, the excess of nearly 0.03 mg. per culture found being accounted for as impurities in the reagents, most of it probably being derived from the glucose.

Final $P_H$ determinations showed a slight increase in the H-ion concentrations, especially in the cultures in the acid end of the series. This was probably due, as we have shown elsewhere, to an unequal absorption of the ions of $NH_4NO_3$. The final iron tests revealed a marked disappearance of soluble iron in the most acid culture where considerable growth had occurred. In the most alkaline cultures, where no trace of the alga was observed, there was still considerable iron in solution, although apparently some had been lost, probably by precipitation.

The dry weights of the crops are plotted in fig. 1. Growth was fairly uniform at H-ion concentrations from $P_H$ 5.1 to 6.9, represented on the graph by an approximately straight line. From $P_H$ 6.9 to 8.0 the growth declined rapidly, as indicated by the steep pitch of this part of the curve. In solutions more alkaline than $P_H$ 8.0 no trace of growth was perceptible, the alkaline limit therefore being approximately 8.3. The decrease in the rate of growth in the

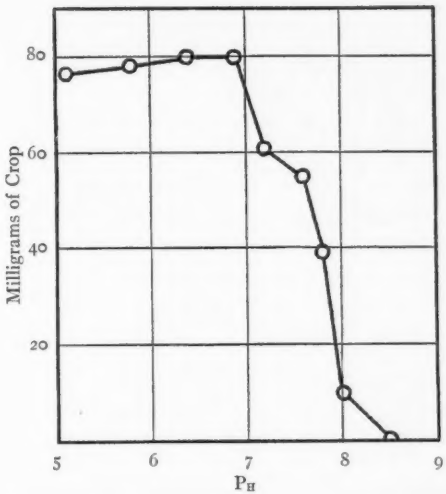


FIG. 1.—Growth-$P_H$ curve, Experiment 1; alkaline limit for growth of *Chlorella*

alkaline cultures of this series cannot be attributed to unavailability of iron, as quantitative tests on these solutions showed that the bulk of the original iron was still present in soluble form.

Aside from the alkaline limit for growth as established by this curve, another feature of interest is the complete absence of any sharp maximum. There is some indication that growth is better at $P_H$ 6.5 than at $P_H$ 5.0 or 5.5, but the differences are not significant. This flat part of the curve corresponds closely to the portion of the curve between $P_H$ 5.0 and 7.0 in the experiment previously reported (**2**, fig. 5).

### Second experiment

The first experiment was repeated, using exactly the same solutions and procedure. The initial $P_H$ determinations showed that the values of the first experiment had been very nearly duplicated, the only wide discrepancies being in the cultures more alkaline than $P_H$ 8.2, where somewhat higher $P_H$ values were recorded than in the previous cases. This can be accounted for by the interval of time

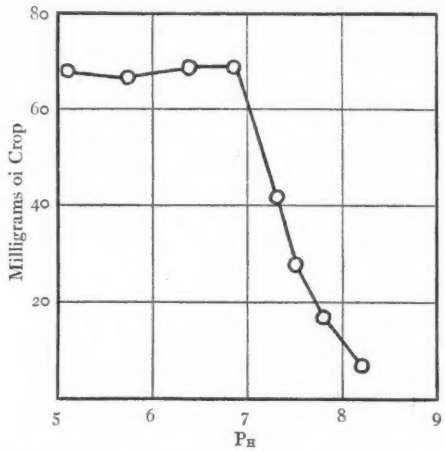


FIG. 2.—Growth-$P_H$ curve, Experiment 2; alkaline limit for growth of *Chlorella*

elapsing between mixing the solutions and making the $P_H$ determinations in the two cases. In the first experiment this time amounted to about 48 hours, during which period the most alkaline cultures no doubt absorbed sufficient $CO_2$ from the air to reduce the $P_H$ values somewhat. In the second experiment the $P_H$ determinations were made within 4 hours after mixing the solutions, which precluded much change in reaction due to the absorption of $CO_2$. These cultures are beyond the limit of growth, however, so that the discrepancies do not affect the results obtained in the earlier experiment.

The detailed data obtained in the second experiment are presented in table II, and the dry weights of the crops are plotted in

fig. 2. The final $P_H$ tests on the culture solutions showed, as in the previous experiment, that the H-ion concentrations of the solutions had increased somewhat during growth of the organism, especially in the acid end of the $P_H$ range. Qualitative tests demonstrated the presence of considerable soluble iron in all of the culture solutions. The $P_H$ growth curve is essentially like that of the first experiment, the point of decline being the same in both cases, as well as the limit for growth. The slope of the curve in the alkaline end of the series in the second experiment appears to be somewhat less steep than in

TABLE II

DATA FROM SECOND EXPERIMENT

| CULTURE NO. | $P_H$ INITIAL | INITIAL Fe PER CULTURE (MG.) | $P_H$ FINAL | | | FINAL Fe (QUALITATIVE TEST) | DRY WEIGHT OF CROP (MG.) | | | AVERAGE CROP (MG.) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | | A | B | C | |
| 1..... | 5.1 | 0.055 | 4.6 | 4.7 | 4.5 | + | 63.2 | 69.8 | 71.0 | 68.0 |
| 2..... | 5.75 | 0 | 5.5 | 5.5 | 5.5 | ++ | 63.3 | 67.7 | 69.3 | 66.8 |
| 3..... | 6.4 | 0 | 6.1 | 6.1 | 6.1 | ++ | 66.4 | 70.1 | 69.9 | 68.8 |
| 4..... | 6.85 | 0 | 6.6 | 6.7 | 6.7 | ++ | 66.2 | 70.2 | 69.9 | 68.8 |
| 5..... | 7.3 | 0 | 7.3 | 7.3 | 7.3 | ++ | 38.6 | 41.2 | 45.8 | 41.9 |
| 6..... | 7.5 | 0.055 | 7.6 | 7.5 | 7.5 | ++ | 25.4 | 30.1 | 28.6 | 28.0 |
| 7..... | 7.8 | 0 | 7.8 | 7.8 | 7.8 | ++ | 20.5 | 16.4 | 14.2 | 17.0 |
| 8..... | 8.2 | 0.055 | 8.2 | 8.1 | 8.1 | ++ | 7.2 | 7.2 | 6.5 | 7.0 |
| 9..... | 9.3 | 0 | 0 | 0 | 8.4 | ++ | ..... | ..... | ..... | 0 |
| 10..... | >9.75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11..... | >9.75 | 0.050 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

> = $P_H$ greater than 9.75 (alkaline limit of Thymol Blue).

the previous series. The absence of a sharp maximum for growth is again clearly evident, as well as the absence of any indication of a double maximum characteristic of some cases of $P_H$ growth curves.

## Discussion

In the final experiment (Experiment 6) of our earlier paper and in the two experiments reported here, it was conclusively demonstrated that soluble iron was present and presumably available in all of the culture solutions regardless of the $P_H$. If the data from these three experiments are plotted on the same scale, the graph shown in fig. 3 is obtained. In this figure the maximum growth obtained in each experiment was taken as 100 and the rest of the data plotted accordingly. One exception to this procedure was made in the case

of Experiment 6 of our first paper, the three points representing growth in the most alkaline cultures of this series being left out of consideration. This curve gives an interesting picture of the relation of H-ion concentration to the growth of *Chlorella* sp.

The acid limit for growth as previously reported is close to $P_H$ 3.4. This has been demonstrated in a number of experiments. The alka-

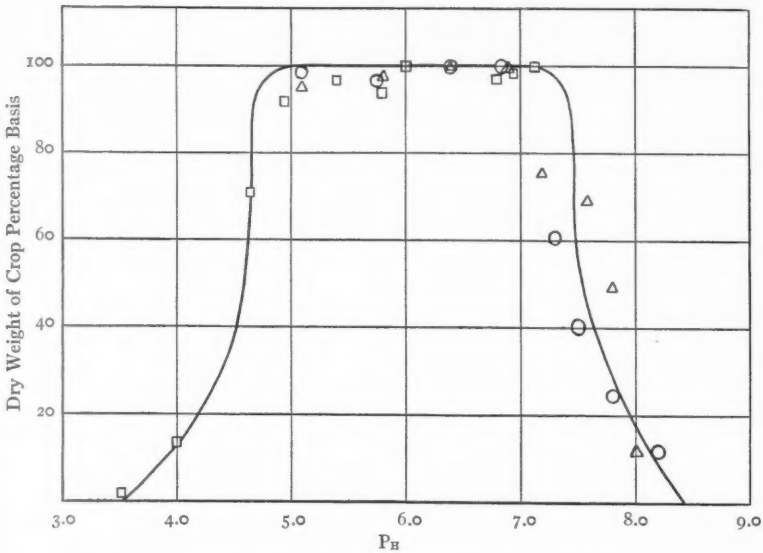


FIG. 3.—Composite growth-$P_H$ curve prepared from data of three separate experiments, showing entire relation of $P_H$ to growth of *Chlorella:* circles, Experiment 1; triangles, Experiment 2; squares, Experiment 6 of previous paper (2).

line limit for growth is about $P_H$ 8.4 according to the data presented in this paper. There appears to be no definite high point or maximum; instead the curve ascends rapidly from both the acid and alkaline limits to a region lying between a $P_H$ of 4.6 to a $P_H$ of 7.0, in which the rate of growth is quite uniform.

Two suggestions might be offered to explain the "flat" portion of this curve. First, that the organism is not very sensitive to changes in H-ion concentration within this range, and second, that under the conditions of the experiment some other factor besides

H-ion or OH-ion concentration is limiting between $P_H$ 4.6 and 7.0. At either end of the "flat" portion of the curve, however, a very slight difference in H-ion concentration on the one hand or in OH-ion concentration on the other will have a marked influence, since the curve drops off so steeply to the limits for growth on either side. As regards the nature of the factor or factors which may be limiting for growth in the region between $P_H$ 4.6 and 7.0, it might be pertinent to mention certain results of investigations soon to be published by the writers on the iron requirement for *Chlorella*. It was found in these studies that, other conditions being the same, at H-ion concentrations near $P_H$ 7.0, rather large amounts of iron were necessary for maximum growth, amounts much greater than used in the experiments reported in this paper. It is possible, therefore, that by increasing the iron content in this region the flat part of the curve would disappear.

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# NEW SPECIES AND VARIETIES OF CHLOROPHYCEAE[1]

L. H. TIFFANY

(WITH PLATE IX)

During the spring and early summer of 1926, the writer made numerous collections of algae along the Wabash River in southeastern Illinois and southwestern Indiana, which contained some species and varieties of *Oedogonium* and a species of *Spirogyra* apparently not previously described. The summer before Professor G. E. NICHOLS of Yale University had sent me a collection of algae from the Michigan Biological Station, Cheboygan, in which appeared an undescribed *Oedogonium*. This latter material was collected by Miss ALMA ACKLEY in the vicinity of Douglas Lake, Michigan, in August 1924. The following is a description of each of these forms, together with notes on distinguishing characteristics, relationship to other species, and time and place of collection. A Latin as well as an English diagnosis is given.

**Spirogyra wabashensis,** nov. sp.

Cellulis vegetativis 40–50 $\mu \times 120$–400 $\mu$, dissepimentis planis; chromatophoris 2–4, anfractibus arctis 0.5–4.5; cellulis fructiferis, singulis vel binis inter cellulas vegetativas distributis, inflatis; tubo conjugationis ex cellula mascula emisso; zygosporis ellipsoideis, 56–76 $\mu \times 110$–150 $\mu$, membrana media reticulata, lutea.

Vegetative cells 40–50 $\mu \times 120$–400 $\mu$, with plane end walls, chromatophores 2–4, making 0.5–4.5 turns; fruiting cells inflated, single or in groups of two alternating with vegetative cells; conjugating tube formed by the male cell; zygospore ellipsoid, 56–76 $\mu \times 110$–150 $\mu$, median spore wall reticulate, yellow.

In the genus *Spirogyra* there have been described to date seven species and one variety in which the conjugating tube is formed wholly or nearly so by male cells. These are *S. punctata* Cleve, *S. punctata* Cleve var. *major* Hirn, *S. punctiformis* Transeau, *S. reflexa* Transeau, *S. micropunctata* Transeau, *S. rugu-*

[1] Paper no. 173 from the Department of Botany, Ohio State University.

*losa* Iwanof, *S. hydrodictya* Transeau, and *S. conspicua* Gay; *S. wabashensis* makes the eighth. The new species is distinguished from all these by its dimensions, number of chromatophores, and its beautifully reticulated median zygospore wall. One particularly noticeable feature of the species is the quite regular alternation of fruiting and vegetative cells, instead of the more usual series arrangement in conjugation. The chloroplasts in the sterile cells of the conjugating filaments are often much distorted and disintegrated, thus giving the appearance of a single chloroplast, or a chloroplast occupying a small portion of the cell length. The species was collected in a pool along the Big Four Railway, 3.5 miles south of Brownsville, Illinois, on May 14 and 20, 1926. Type in herb. L. H. T. collections nos. 21 and 42 (Illinois).—Fig. 1.

**Oedogonium wabashense,** nov. sp.

O. diocium, nannandrium, gynandrosporum; oogoniis singulis vel 2–3 continuis, ellipsoideis vel ovoideis, saepe terminalis, operculo apertis, circumscissione suprema, operculo saepe deciduo; oosporis eadem forma ac oogoniis (rarius globosis), complentibus vel non complentibus, membrana laevi; androsporangiis 1–3 cellularibus, subepigynis; cellulis vegetativis evidenter capitellatis; cellula fili basali forma, ut vulgo, elongata; nannandribus in oogoniis sedentibus; antheridio 1–? cellulari; crass. cell. veg. 12–20 μ, long. 36–64 μ; crass. oogon. 36–42 μ, long. 44–60 μ; crass. oospor. 34–38 μ, long. 40–55 μ; crass. cell. andros. 12–16 μ, long. 10–20 μ; crass. stip. nanndr. 12–16 μ, long. 24–40 μ; crass. cell. antherid. 7–12 μ, long. 6–10 μ; crass. cell. basal. 18–20 μ, long. 40–64 μ.

O. dioecious, nannandrous, gynandrosporous; oogonia single or in groups of 2–3, ellipsoid or ovoid, often terminal, operculate, division at the upper extremity of the oogonium, lid often deciduous; oospores of the same form as the oogonia (rarely globose), which they completely fill or not, walls smooth; androsporangia 1–3-celled, subepigynous; vegetative cells evidently capitellate; basal cells elongate; dwarf males on the oogonia; antheridia 1–?-celled; vegetative cells 12–20 μ×36–64 μ; oogonia 36–42 μ×44–60 μ; oospores 34–38 μ×40–55 μ; androsporangial cells 12–16 μ×10–20 μ; dwarf male stipes 12–16 μ×24–40 μ; antheridial cells 7–12 μ×6–10 μ; basal cells 18–20 μ×40–64 μ.

This species evidently belongs in the group with *O. obtruncatum* Wittrock, *O. praticolum* Transeau, and *O. supremum* Tiffany. It differs from the first in having smaller fruiting cells and pluricellular dwarf males; from the second and

third it is distinguished by its gynandrosporous habit and smaller dimensions throughout. The lid of the non-terminal oogonium sometimes is much distorted, and gives one the impression of a superior division. When in this condition it has the general appearance of *O. ciliatum* (Hass.) Pringsh., differing however in size, shape of fruiting cells, absence of a terminal seta, and in being capitellate. It was collected in the bed of the old River de Shea near the Wabash River, 15 miles south of Vincennes, Indiana, May 26, 1926. Type in herb. L. H. T. collections nos. 47, 50, 52, 53 (Illinois).—Figs. 3, 4, 5.

OEDOGONIUM HOWARDII West, var. **minor,** nov. var.

Var. omnibus partibus gracilioribus: crass. cell. veg. 7–9 μ, long. 27–48 μ; crass. oogon. 26–29 μ, long. 28–36 μ; crass. oospor. 24–26 μ, long. 24–26 μ; crass. cell. antherid. 7–8 μ, long. 10–12 μ; crass. cell. basal. 12–14 μ, long. 10–12 μ.

Smaller than the type; vegetative cells 7–9 μ×27–48 μ; oogonia 26–29 μ×28–36 μ; oospores 24–26 μ×24–26 μ; antheridial cells 7–8 μ×10–12 μ; basal cells 12–14 μ×10–12 μ.

The variety is distinguished from the type only by its smaller dimensions. The occasional subspherical basal cells and the usual shape of the oogonium, together with its much smaller size throughout, make it easily separable from *O. latiusculum* Tiffany. It was collected in a gravel pit pond 3 miles east of Pinkstaff, Illinois, May 28, 1926. Type in herb. L. H. T. collections nos. 55 and 56 (Illinois).—Figs. 6 and 7.

OEDOGONIUM BRAUNII Kuetz.; Pringsh. var. **Zehneri,** nov. var.

Var. omnibus partibus crassioribus, oosporis ovoideis; crass. cell. veg. 12–24 μ, long. 34–72 μ; crass. cell. suffult. 21–32 μ, long. 48–52 μ; crass. oogon. 40–50 μ, long. 48–60 μ; crass. oospor. 34–44 μ, long. 36–50 μ; crass. stip. nanndr. 8–10 μ, long. 16–24 μ; crass. cell. antherid. 7–8 μ, long. 8–12 μ.

Somewhat larger than the type in all dimensions; vegetative cells 12–24 μ×34–72 μ; suffultory cells 21–32 μ×48–52 μ; oogonia 40–50 μ×48–60 μ; oospores 34–44 μ×36–50 μ; dwarf male stipes 8–10 μ×16–24 μ; antheridial cells 7–8 μ×8–12 μ.

The habit of this variety is clearly that of *O. Braunii* Kuetzing; Pringsheim, differing in its larger size, particularly of the fruiting cells, and its differently shaped oospores. The dwarf male stipe is sometimes 4–5-celled and much elongated, having somewhat the appearance of another species of *Oedogonium* epiphytic upon it. In dimensions it is near *O. gallicum* Hirn, but the latter does not have swollen suffultory cells. It is larger than *O. Braunii* var. *hafniense*

(Hallas) Hirn. Collected from a pool along the Chicago and Eastern Illinois Railway, 3 miles south of Vincennes, Indiana, May 26, 1926. Type in herb. L. H. T. collections no. 44.—Fig. 2.

OEDOGONIUM ARESCHOUGII Wittr. var. **americanum,** nov. var.

Var. idioandrospora, oogoniis minoribus, androsporangiis ad 11-cellularibus; crass. cell. veg. 8–12 μ, long. 40–80 μ; crass. oogon. 29–36 μ, long. 26–40 μ; crass. oospor. 23–26 μ, long. 22–26 μ; crass. cell. andros. 7–9 μ, long. 7–11 μ.

Idioandrosporous, oogonia smaller, androsporangia to 11-celled; vegetative cells 8–12 μ×40–80 μ; oogonia 29–36 μ×26–40 μ; oospores 23–26 μ×22–26 μ; androsporangial cells 7–9 μ×7–11 μ.

This form clearly belongs in the "Areschougii group," but its dimensions and idioandrosporous habit separate it from the type and from f. *robustum* Hirn. It was collected from a small lake near De Soto, Illinois, May 15, 1926. Type in herb. L. H. T. collections nos. 27, 28, and 29 (Illinois).—Figs. 8 and 9.

**Oedogonium michiganense,** nov. sp.

O. dioicum, nannandrium, gynandrosporum; oogoniis singulis vel 2–7 continuis, globosis vel ellipsoideo-globosis (rarius subglobosis), operculo apertis, circumscissione suprema; oosporis globosis, oogoniis complentibus vel non complentibus, membrana triplici: episporio (in latere exteriore) laevi, mesosporio longitudinaliter costato (in sectione optica transversali undulato), costis crenulatis, interdum anastomosantibus, in medio oosporae c:a 12–24, endosporio laevi; cellulis suffultoriis tumidis; cellulis vegetativis leviter capitellatis; cellula fili basali forma, ut vulgo, elongata; cellula terminali, quae interdum est oogonium, apice obtusa; nannandribus paullum curvatis in cellulis suffultoriis sedentibus, antheridio interiore; crass. cell. veg. 12–24 μ, long. 80–160 μ; crass. cell. suffult. 32–48 μ, long. 64–80 μ; crass. oogon. 50–64 μ, long. 50–80 μ; crass. oospor. 44–60 μ, long. 44–60 μ; crass. cell. androsp. 16–20 μ, long. 16–20 μ; crass. nannandr. 14–20 μ, long. 40–56 μ; crass. cell. basal. 18–20 μ, long. 70–100 μ.

O. dioecious, nannandrous, gynandrosporous; oogonia single or in groups of 2–7, globose to ellipsoid-globose (rarely subglobose), operculate, division at the upper extremity of the oogonium; oospore globose, filling the oogonium or not, outer spore wall smooth, middle

wall with 12–24 crenulate, sometimes anastomosing, longitudinal ribs, inner wall smooth; suffultory cells enlarged; vegetative cells broadly capitellate; basal cells elongate; terminal cells, occasionally oogonia, apically obtuse; dwarf males, a little curved, on suffultory cells, antheridia interior; vegetative cells 12–24 $\mu \times 80$–$160\ \mu$; suffultory cells 32–48$\mu$ $\times 64$–$80\ \mu$; oogonia 50–64 $\mu \times 50$–$80\ \mu$; oospores 44–60 $\mu \times 44$–$60\ \mu$; androsporangial cells 16–20 $\mu \times 16$–$20\ \mu$; dwarf males 14–20 $\mu \times 40$–$56\ \mu$; basal cells 18–20 $\mu \times 70$–$100\ \mu$.

The position of the operculum, the markings of the oospore, the enlarged suffultory cells, and the capitellate vegetative cells present a combination of characteristics that necessitates a new species among the operculate forms of the genus. It was collected by Miss ALMA ACKLEY in small pools of Nichols' Bog and Smith's Bog in the vicinity of Douglas Lake, Cheboygan County, Michigan, during August 1924. Type in Nichols' collections nos. 202 and 212 and in L. H. T. Michigan collections nos. 1–6.—Figs. 10, 11, 12.

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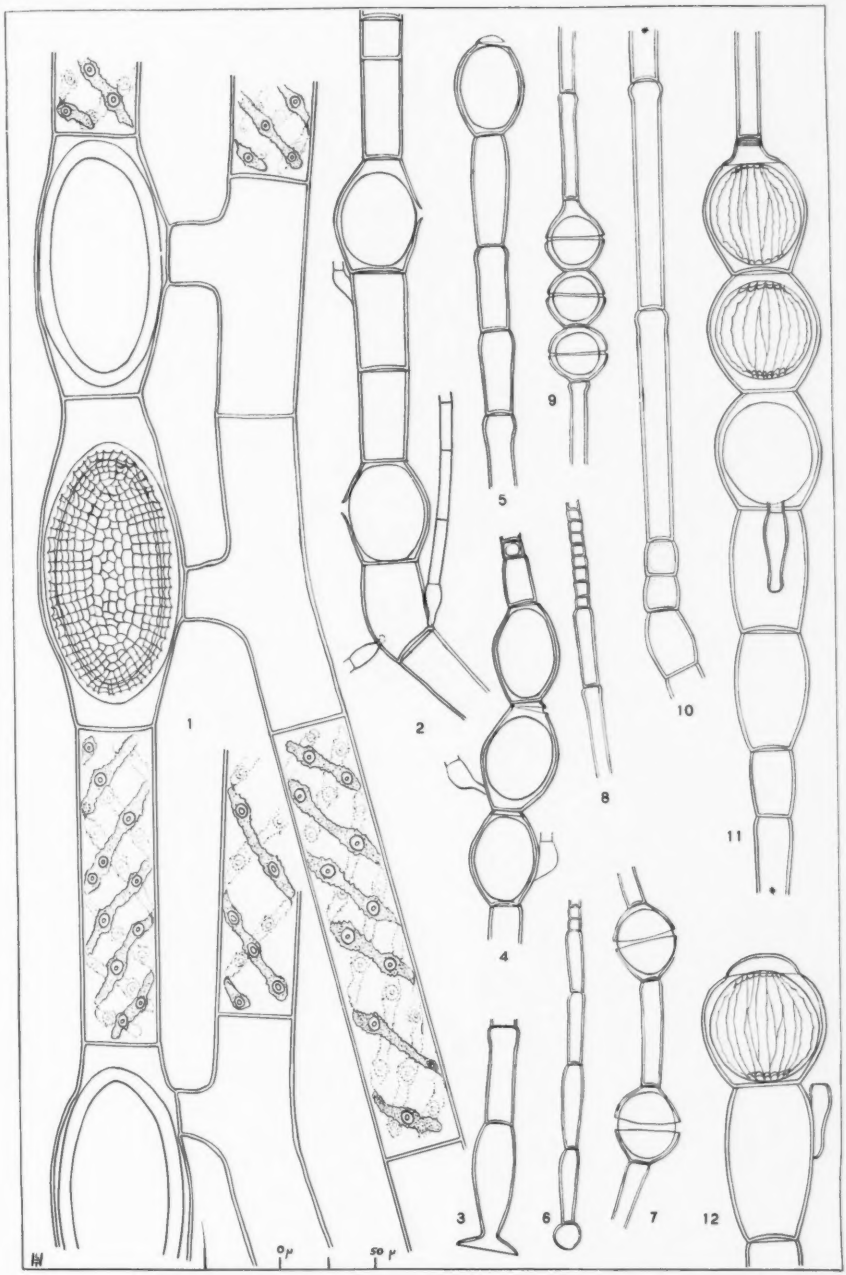
## EXPLANATION OF PLATE IX

FIG. 1.—*Spirogyra wabashensis*, nov. sp.
FIG. 2.—*Oedogonium Braunii* Kuetz.; Pringsh. var. *Zehneri*, nov. var.
FIGS. 3–5.—*Oedogonium wabashense*, nov. sp.
FIGS. 6, 7.—*Oedogonium Howardii* West var. *minor*, nov. var.
FIGS. 8, 9.—*Oedogonium Areschougii* Wittr. var. *americanum*, nov. var.
FIGS. 10–12.—*Oedogonium michiganense*, nov. sp.




TIFFANY on CHLOROPHYCEAE

# EFFECT OF REACTION OF SOLUTION ON GROWTH OF ALFALFA[1]

A. R. C. HAAS

(WITH THREE FIGURES)

Although an extensive literature is accumulating in regard to the effect of reaction on the growth of alfalfa, there is only partial agreement between the results of several recent investigations (1, 3, 4, 5). Some have found slightly alkaline solutions favorable to the growth of alfalfa, while others have found that approximately neutral or slightly alkaline culture solutions may have an injurious effect. Under field conditions alfalfa seems to do very well on soils which are slightly to moderately alkaline. It is therefore inconsistent with observations in the field that alfalfa should be injured by a neutral or slightly alkaline medium, or else our knowledge of what constitutes the actual reaction of the soil solution is seriously at fault. Lack of agreement between the results of culture solution tests and those of field trials may in part be due to the type of cultures and culture solutions employed. Solution cultures are ordinarily considered to be more reliable than sand or soil cultures in regard to the maintenance of the desired reaction. This may or may not be true, depending considerably upon the type of solution employed, the nature of the acid or alkali used in maintaining a given reaction, the frequency of renewal of the culture solution and of the desired $P_H$, as well as many other factors. By the use of controlled solution cultures, the writer has obtained results on the effect of reaction on the growth of alfalfa which are in harmony with those obtained on soils in the field, where the actual reaction is considered to be slightly to moderately alkaline.

The solution employed was a modified Hoagland solution in which double the usual amount of calcium nitrate was used and to which was added a trace of many ions not commonly used in solution cultures. Such a solution gives very favorable growth when

[1] Paper no. 154, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

used with solution cultures of citrus. It has the following composition expressed as parts per million:

| Na | K | Ca | Mg | $NO_3$ | Cl | $SO_4$ | $PO_4$ |
|---|---|---|---|---|---|---|---|
| 7 | 185 | 318 | 54 | 1214 | 10 | 216 | 105 |

with traces of ferric tartrate, aluminum sulphate, potassium iodide, titanium sulphate, potassium bromide, strontium nitrate, lithium nitrate, manganese sulphate, boric acid, and ammonium nitrate (**2**).

TABLE I

GROWTH OF ALFALFA IN MODIFIED HOAGLAND'S SOLUTION; FIRST EXPERIMENT

TOTAL FRESH WEIGHT (GM.) OF ENTIRE PLANTS IN EACH SERIES

| SERIES | $P_H5$ | $P_H6$ | $P_H7$ | $P_H8$ |
|---|---|---|---|---|
| NaOH..... | 8.0 | 9.0 | 12 | 11.5 |
| $Ca(OH)_2$..... | 4.5 | 7.5 | 8 | 9.5 |

TABLE II

GROWTH OF ALFALFA IN MODIFIED HOAGLAND'S SOLUTION; SECOND EXPERIMENT

TOTAL FRESH WEIGHT (GM.) OF ENTIRE PLANTS IN EACH SERIES

| SERIES | NO. OF PLANTS | $P_H5$ | $P_H6$ | $P_H7$ | $P_H8$ |
|---|---|---|---|---|---|
| NaOH..... | 65 | 17.5 | 14.5 | 21 | 26 |
| KOH..... | 65 | 14.0 | 16.5 | 18 | 21 |

Distilled water containing small amounts of ferric tartrate was added to each culture every day. The solution had a $P_H$ of 5.0–5.2, and was contained in enameled pails of 9 liter capacity, with covers of heavily tinned iron.

The alfalfa seeds were germinated in sand to which was added the modified Hoagland's solution, and were grown in the sand until the first leaves above the cotyledonary ones had developed. The seedlings were then washed free from most of the adhering sand, inserted

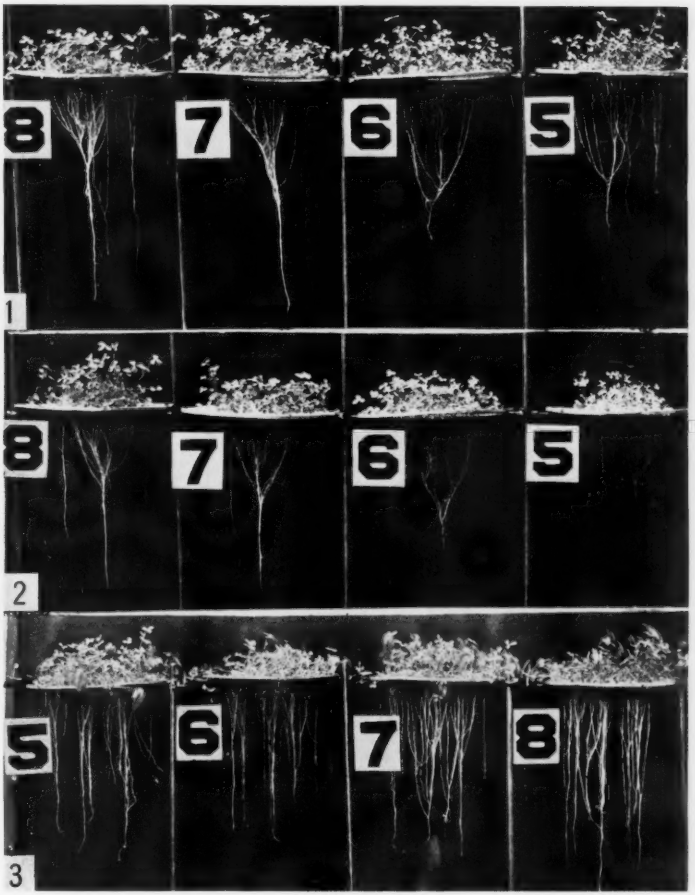



FIG. 1.—Growth of alfalfa in culture solutions maintained at various $P_H$ values by addition of NaOH.

FIG. 2.—Growth of alfalfa in culture solutions maintained at various $P_H$ values by addition of $Ca(OH)_2$.

FIG. 3—Growth of alfalfa in culture solutions maintained at various $P_H$ values by addition of KOH.

into small holes in the covers, and left to support themselves without the use of plugs. The $P_H$ value of the solution in the pails in each series was brought to 5, 6, 7, or 8 by additions of alkali two or three times daily as required. The culture solutions were renewed every 10 to 14 days.

FIRST EXPERIMENT.—On April 8, thirty-six alfalfa seedlings were placed in each solution culture and the $P_H$ was brought to 5, 6, 7, and 8 by means of NaOH in one series and by means of $Ca(OH)_2$ in a second series. The cultures were continued until May 6; during the culture period the solutions were renewed three times. Table I gives the fresh weights of the plants in the first series, and figs. 1 and 2 show the growth in the two series. It is evident that in both series the growth was better at $P_H$ 7 and 8 than at $P_H$ 5 and 6.

SECOND EXPERIMENT.—On June 10 another series similar to the NaOH series was begun with alfalfa seedlings, but at higher temperatures because of the advancing summer. The plants were grown until June 29. On June 16 a series was begun in which KOH was employed in maintaining the desired reaction, and the cultures were continued until July 6. Fig. 3 shows the growth obtained, which is typical of both the series. The tops of the plants were 5–6 inches high but became wilted during the long photographic exposure in the hot glasshouse. Table II gives the fresh weights. As in the first experiment, growth was better in the neutral and alkaline solutions than in the acid ones.

## Summary

Alfalfa seedlings were grown in Hoagland's solution modified by doubling the calcium nitrate, adding traces of certain other elements, and adjusting the $P_H$ with NaOH, $Ca(OH)_2$, or KOH. Neither approximate neutrality nor moderate alkalinity appeared to be unfavorable to growth. The average growth was least at $P_H$ 5 and greatest at about $P_H$ 8.

CITRUS EXPERIMENT STATION
RIVERSIDE, CALIF.

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# CURRENT LITERATURE

## BOOK REVIEWS

### Cytology of flowering plants

A comprehensive treatment of the cytology of gymnosperms and angiosperms has long been needed by investigators in this field. The *Morphology of angiosperms* by COULTER and CHAMBERLAIN treated the whole subject of morphology, but the date of its publication is 1903, whereas most of the critical cytological literature, and nearly all of the cytology of genetics has developed since that time. The third edition of the *Morphology of gymnosperms*, by the same authors, brings the literature up to 1917, but here again the treatment covers the whole field of morphology. Consequently, the recent book by SCHÜRHOFF[1] covers a little occupied field. It does not pretend to present the results of the author's own investigations, although these have been extensive; but is written in textbook style, and presents a critical and well organized summary of the literature up to 1926. The presentation is scholarly and unprejudiced, and work from other countries is given adequate recognition.

The first part, on general cytology, deals with rather strictly nuclear phenomena and gametophytes. It includes a discussion of the significance cytological features may have in systematic botany. In the second part the cytology of the various groups of seed plants is treated separately, with copious references to literature. In many families the chromosome numbers are given, and features which may be of value in any phylogenetic treatment of taxonomy are emphasized. It is well known that most cytological investigations have been made upon fixed and stained material. Studies on living material are also presented, but the author concludes that such studies have added little to our knowledge, except to confirm the results obtained by microtechnical methods.

The haploid generation has not been used much in the taxonomy of higher plants, and keys based upon cytological features would be hard to apply; but the comprehensive treatment of the haploid generation assembles and organizes the data so that they can be used in taxonomic problems, so far as they are concerned with phylogeny at least.

There are more than 2000 references to literature, and the volume undoubtedly will facilitate investigation in both the morphology and cytology of seed plants.—C. J. CHAMBERLAIN.

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[1] SCHÜRHOFF, P. N., Die Zytologie der Blüthenpflanzen. 8vo. pp. xi+792. *figs. 282*. Stuttgart: Ferdinand Enke. 1926.

**Fungi of Middle Europe**

Number 1 and part of number 2 (the colored plates only) of Volume I of a new work on fungi[2] have made their appearance. This volume is to be devoted to the Boletaceae. This series of publications is under the auspices of the German Society of Mycology, the German Botanical Society, and the German Society for Teaching Biology, and is intended to cover the entire field of mycology, each group of fungi being assigned to a specialist for monographic presentation. So far as possible, the fungi are to be illustrated in natural colors, in natural size, and various species are to be represented by more than one specimen, so that both the range in form and color, as well as the various stages in the life history of the species will be given.

Number 1, which is a representative of the 20–25 numbers of Volume I, contains two colored plates, two plates in black and white, and four pages of text. The colored illustrations are beautifully done. The text consists of description of the plates, and in addition presents material under the following headings: original and modern detailed description and diagnosis of the species; economic and toxicological aspects; possible confusion with other species; microscopic studies; habitat and geographic distribution; history; bibliography.

The work is excellent and should prove exceedingly interesting and useful to American specialists and amateurs in the field of mycology. It is a matter of regret that a similar work on North American fungi is not under way.—G. K. K. LINK.

## NOTES FOR STUDENTS

**Suction force of plant cells.**—In a lengthy paper BLUM[3] continues the discussion of suction force of plant cells, using numerous alpine species. By means of URSPRUNG'S simplified method he found very high suction forces, so that the direct readings of BERKELEY and HARTLEY, and of FRAZER and MYRIK were used for the determination of exact volumes. Aerial tissues were always found to have a higher suction force than root tissues. *Campanula glomerata* was found to have a suction force in its leaves of 44.5 atmospheres during the summer. The values varied with season, rainfall, and other variable factors, as would be expected. Floral parts also had high values in some cases; thus the petals of *Lotus corniculatus* showed 34.5 atmospheres, but *Pinguicula alpina* had a low value for its flowers, only 2 atmospheres. Of the factors modifying suction force of cells, soil moisture has the chief influence. When the soil is no longer very moist, atmospheric humidity changes modify the suction force rather strongly. With the diurnal changes in saturation deficit the suction force keeps

[2] Die Pilze Mitteleuropas, under the editorship of KNIEP, H. (Berlin), CLAUSSEN, P. (Marburg), and BASZ, J. (Stuttgart). Leipzig: W. Klinghardt. Band I. no. 1. 1926. M. 4.00.

[3] BLUM, G., Untersuchungen über die Saugkraft einiger Alpenpflanzen. Beih. Bot. Centralbl. **43**:1–100. 1926.

pace in a general way; and dry and moist habitats always have high or low average suction force values.

As standards of reference the behavior of *Taraxacum* and *Bellis* was taken, and other plants compared with them. Thus the limits of variability of suction force of one species could be compared with another in the same and different habitats. Some exceptional plants were noted, such as *Ranunculus aconitifolius*, which occupied moister areas, yet always had very high suction values as compared with the standard plants. Also the xerophile *Thymus serpyllum*, occupying drier habitats, had consistently lower values than the standards. Ecological groups were compared, and lower average values were found for the moisture indicators, fertility indicators, and shade plants. Higher average values were found for the arid indicators, calcicoles, poor land indicators, and sun plants. These suction force studies show in a different way, by plasmolytic means, just what HARRIS' studies of freezing point depressions have shown, a general correspondence of plant cells to the conditions of the habitat.

The reviewer dislikes the term suction force. If we speak of suction at all, it is a suction tension, but it is questionable whether we should use the word suction at all. If a careful analysis of water movement is made, it will always be found that water is the active compound. The cell contents merely provide a medium of lower free water content into which water from regions of greater free water content moves. The pressure in any cell is caused by the free water entering from outside the cell. If the pressure in a street car, when overcrowded with people who try to enter after the car is full, can be called a suction pressure, then the pressures in a cell can be called suction pressure, and the force of entry called a suction tension. URSPRUNG and BLUM have shown how to measure osmotic pressure accurately by the plasmolytic method, a method which has been a very faulty tool until now, and this is an important contribution. The reviewer, however, sees no good reason for using any other term than osmotic pressure in connection with the turgidity of plant cells.—C. A. SHULL.

**Function of iodine.**—All organisms that inhabit the earth use iodine, according to STOKLASA.[4] In forms like bacteria, that respire very rapidly, he believes iodine serves in the oxidase system. Among higher plants, halophytes, hygrophiles, and hydrophytes use the most iodine. Here too the iodine is thought to be related to the respiratory system. Root systems with iodine give off more $CO_2$ than without iodine. This result might be due to more vigorous growth; but STOKLASA points out that in the presence of iron and radium emanation there is much more $CO_2$ output in iodine-rich roots, than in those poor in iodine. Iodine in some way also prevents development of high acidity internally. *Senecio vulgaris*, without iodine added to the soil, showed a $P_H$ of 2.68 and 3.02

[4] STOKLASA, JULIUS, Die physiologische Funktion des Jods beim Bau- und Betriebsstoffwechsel in der chlorophyllhaltigen und chlorophyllosen Zelle. Biochem. Zeitschr. **176**:38–61. 1926.

for aerial parts and roots respectively, while the specimens given iodine showed a $P_H$ of 4.78 in the aerial portions, and 5.1 for the roots. This internal control of H-ion concentration prevents acid depression of enzyme activity, and injury to the chlorophyll apparatus. The chlorophyllous parts are always richer in iodine than the root system. The transformations of energy and materials in general are accelerated in plants having iodine, the basal metabolism being favored by its presence. Since halophytes and other iodine users have more furfuroids in them, STOKLASA thinks the iodine favors the transformation of cane sugar into furfuroids. But this is really arguing that any differences found in plants that use iodine are due to the iodine, a loose type of reasoning of which we have far too much in biology.

Certain plants take up iodine and deposit it in organic form. These plants are referred to as iodine-saturated. The pharmacological usefulness of such plants is pointed out. Potatoes, radishes, and tomatoes can be saturated in this way if iodine fertilizer is added to the soil. Experimenting on himself by eating vegetables grown without iodine, and then with iodinized fruits and vegetables of the same kind, he shows that the acidity of urine is much decreased by an iodine-containing diet. However, inorganic iodine does not give good results. The iodine is needed in organic form. The proper way, then, to get our iodine, is not by using iodized salt, but by growing vegetables on soils furnished with something like 50 ppm of iodine, and using these for food. Whatever may be the needs of plants for iodine, and the exact functions of iodine in the plant body, the need of iodine by animals and man has been fully demonstrated, and the pathological effects of lack of iodine are in part known. STOKLASA's method of applying the dosage is certainly the proper method. Sea foods, and vegetables grown on iodine fertilized soils, would no doubt be valuable in the diet of everybody.—C. A. SHULL.

**Chemical determination of sex.**—A number of workers have been attracted by the claim of MANOILOFF that the sexes can be distinguished by means of a chemical reaction. Essentially his test is based on the idea that alcoholic extracts from female tissues possess a reducing substance, while those from males have an oxidizing effect. The color changes occurring in a mixture of papayotin, dahlia, potassium permanganate, hydrochloric acid, and thiosinamin, to which extract from males or females has been added, are believed to indicate with a high degree of accuracy whether the extracts come from one sex or the other.

The method has been subjected to a critical test by ALSTERBURG and HAKANSSON,[5] who used not only rabbits, sparrows, and fish, but many kinds of plants, hermaphroditic, monoecious, and dioecious. *Magnolia*, *Tulipa*, and *Paeonia* were used as hermaphrodites, *Carpinus*, *Fagus*, and *Alnus* among the monoecious forms, and four species of dioecious willows, *Rhodeola rosea*, *Pela-*

[5] ALSTERBURG, GUSTAF, and HAKANSSON, ARTHUR, Über Manoiloffs Reaktionen und die Möglichkeit, mit Hilfe dieser das Geschlecht zu bestimmen. Biochem. Zeitschr. **176**:251–265. 1926.

*sites albus*, and *Melandrium rubrum* among the latter group. In more than 50 per cent of the cases the reaction failed to indicate the sex. This is a much poorer showing than is claimed by MANOILOFF himself, and by SATINA and coworkers in this country.

They conclude that the MANOILOFF reaction is only quantitative, not qualitative. They claim that there is no enzyme involved, and no sex hormone. The papayotin is held to be useless in the mixture, as the only feature actually involved is the ability of organic substances to reduce, to a greater or less degree, potassium permanganate. The same effects can be produced by purely inorganic substances, as by the MANOILOFF reaction. The dahlia or methyl violet may also have some reducing action, but mainly serve to show whether oxidation or reduction is dominant. If oxidation prevails, the mixture goes colorless. If reduction is greater, a part of the pigment remains to give color as found by MANOILOFF. The permanganate is an oxidizer, and by using a measured amount, one has a measure of the reducing substance in the extract. The rôle of thiosinamin is not very clear, but excess of oxidation medium may be taken care of by this means.

With organic material from many sources, the authors have been quite unable to substantiate MANOILOFF's claim for this method of determining the sex of individual animals or plants.—C. A. SHULL.

**Endosperm activity.**—Using sterile conditions, GRÜNFELD[6] shows that corn endosperm freed of embryo and placed in 10 cc. of water will empty itself of reserves in 35–40 days at 25° C. As it takes a germinating embryo only half as long to do this, the embryo must play some part in the hydrolytic process. It probably excretes some enzyme, and at any rate keeps the products from accumulating and delaying the hydrolysis. In isolated endosperm the rate of hydrolysis rises for 5–7 days to a maximum, maintains this high rate for several days, then falls off during the next 10 days to a low value. Complete cessation of hydrolysis occurs when a sugar concentration of 4–5 per cent in the surrounding water is reached. The medium was found to contain mainly glucose, much maltose, some cane sugar, and traces of fructose, besides an undetermined carbohydrate.

Partly emptied endosperms will reverse the process and fill up with starch if given an aqueous solution mixture of 5 per cent dextrose, 5 per cent cane sugar, 5 per cent dextrine, and the natural hydrolytic products of endosperm hydrolysis. Without these products the reversal does not take place. From the ability to digest itself, and refill itself with starch, GRÜNFELD assumes that the endosperm is alive. It may be, but why should not a non-diffusing diastatic enzyme be able to do the same thing in cells that are not actually living?—C. A. SHULL.

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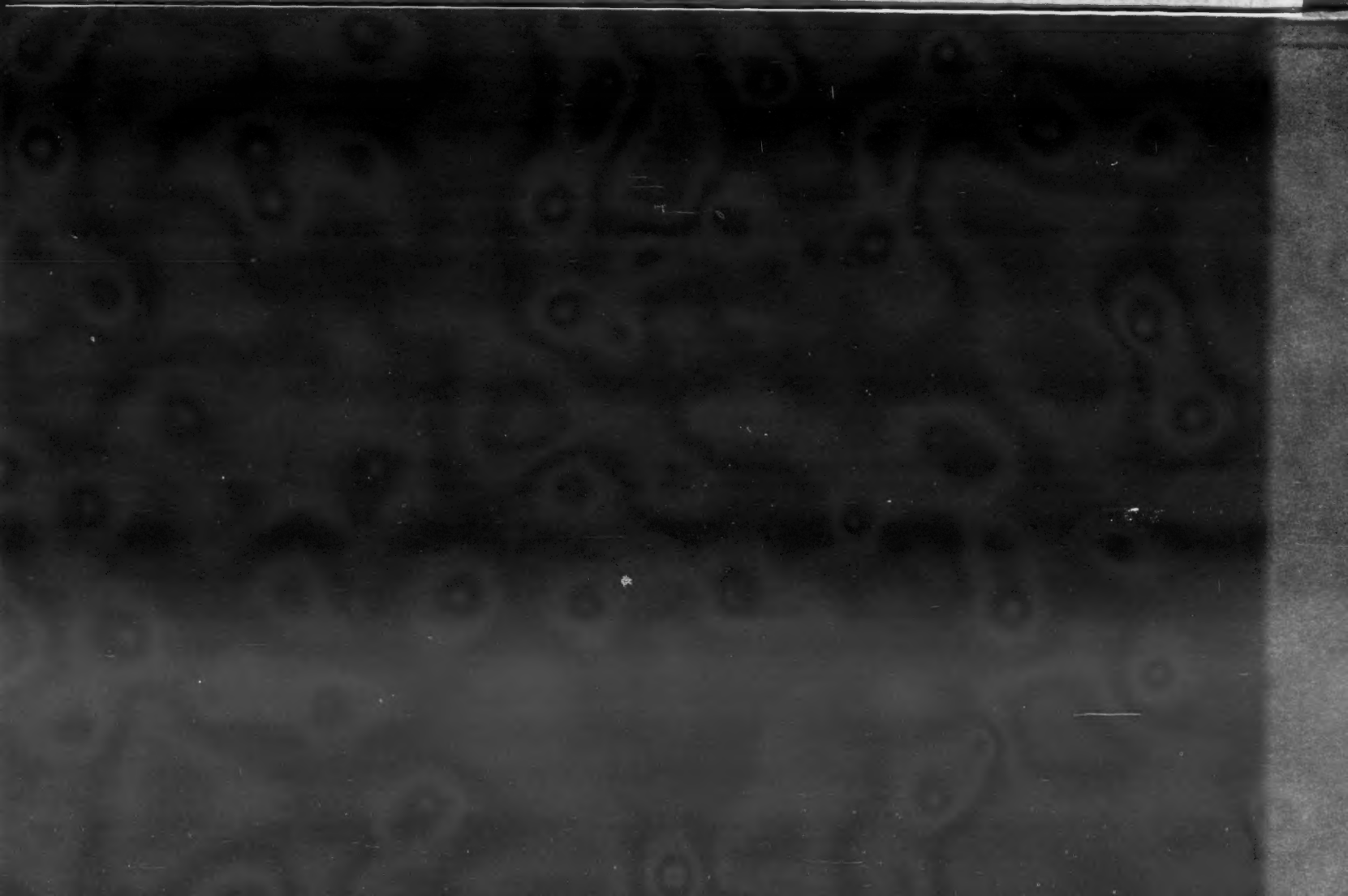
[6] GRÜNFELD, OTTO, Über die Entleerung und Wiederauffüllung isolierter Getreideendosperme, insbesondere von Mais, unter aseptischen Bedingungen. Beih. Bot. Centralbl. 43:167–200. 1926.



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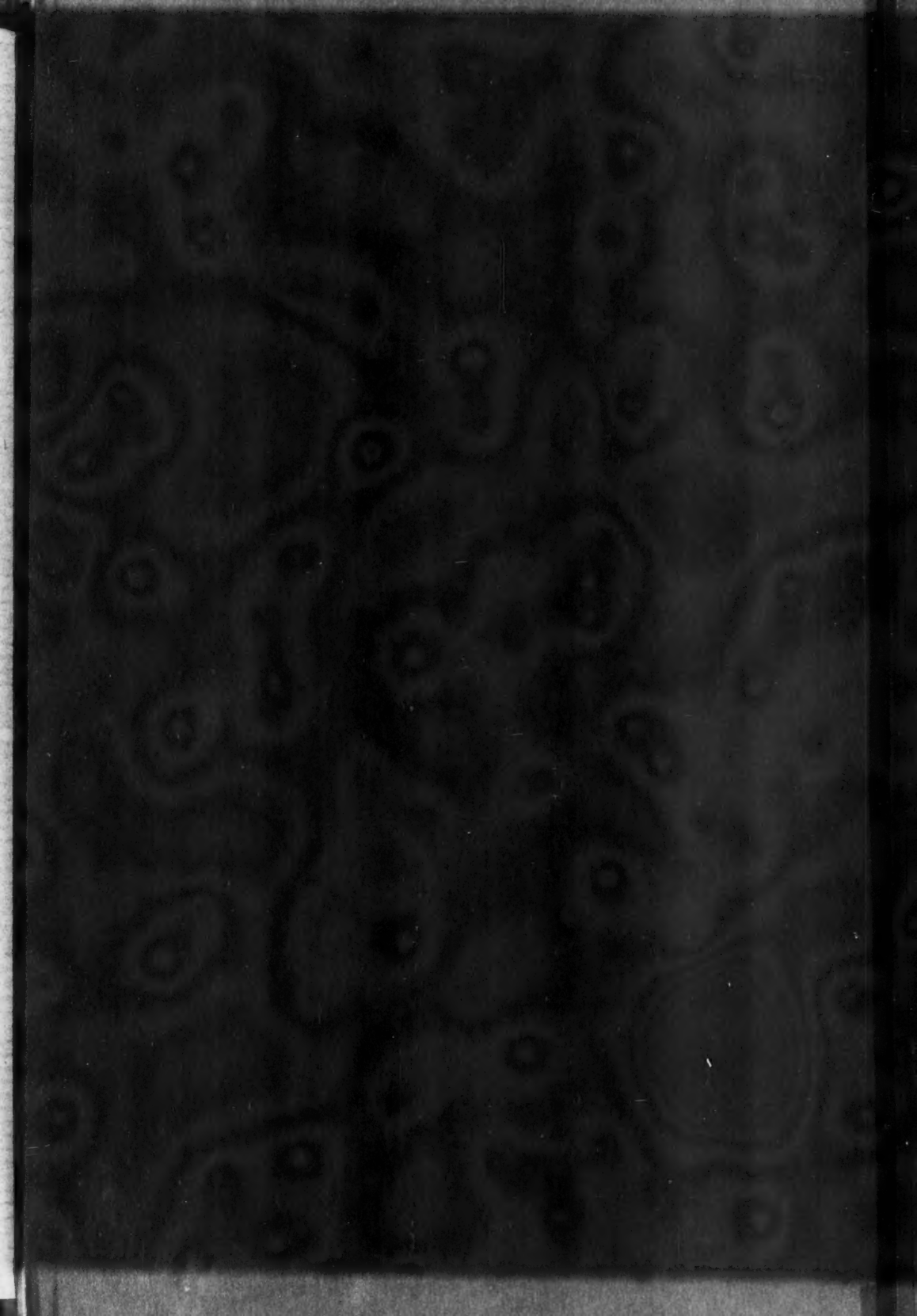
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